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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C07K 14/715, C12N 15/12, 15/63, C07K 16/28 — A2

(11) International Publication Number: **WO 00/34335**

(43) International Publication Date: 15 June 2000 (15.06.00)

(21) International Application Number: PCT/US99/26035

(22) International Filing Date: 3 December 1999 (03.12.99)

(30) Priority Data:
09/205,018 4 December 1998 (04.12.98) US

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, HR, HU, ID, IL, IN, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LU, LV, MA, MD, MG, MK, MN, MX, NO, NZ, PL, PT, RO, RU, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: MAMMALIAN GENES; RELATED REAGENTS

(57) Abstract

Purified genes from a mammalian virus, reagents related thereto including purified proteins, specific antibodies, and nucleic acids encoding the polypeptides are provided. Methods of using said reagents and diagnostic kits are also provided.

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MAMMALIAN GENES; RELATED REAGENTS

FIELD OF THE INVENTION

The present invention pertains to compositions related to proteins which exhibit sequence similarity to TNF receptors which function in controlling activation and expansion of mammalian cells, e.g., cells of a mammalian immune system. In particular, it provides purified genes, proteins, antibodies, and related reagents useful, e.g., to regulate infection, activation, development, differentiation, and function of various cell types, including hematopoietic cells.

BACKGROUND OF THE INVENTION

Apoptosis, programmed cell death, is an important physiological process in the development and homeostasis of multicellular organisms. See, e.g., Schulze-Ostoff, et al. (1998) Eur. J. Biochem. 254:439-459; Wallach, et al. (1998) Current Opin. Immunology 10:279-288; Lockshin, et al. (eds. 1998) When Cells Die: A Comprehensive Evaluation of Apoptosis and Programmed Cell Death; Yuan (1997) Current Opin. Cell Biology 9:247-251; Martin (ed. 1996) Apoptosis and Cancer, Karger; Sluyser (ed. 1996) Apoptosis in Normal Development and Cancer; Baker and Reddy (1996) Oncogene 12:1-9; Ware, et al. (1996) J. Cell. Biochem. 60:47-55; Gregory (ed. 1995) Apoptosis and the Immune Response Wiley and Sons; and Lavin and Watters (eds. 1993) Programmed Cell Death: The Cellular and Molecular Biology of Apoptosis Harwood. Moreover, apoptosis represents a highly efficient defense mechanism against harmful invaders such as viruses, allowing the "clean" disposal of viral proteins and nucleic acids by the infected host. See, e.g., Tschopp, et al. (1998) Current Opin. Genetics and Development 8:82-87. The induction of apoptosis of virus-infected cells is an important host defense mechanism against invading pathogens. Some viruses express anti-apoptotic proteins

that efficiently block apoptosis induced by death receptors. Viral interference with host cell apoptosis leads to enhanced viral replication and may promote cancer.

The discovery of new means to block apoptosis is useful, particularly, where those means may effect blockage of the apoptosis pathways. Moreover, the inability to modulate apoptosis signals prevents control of inappropriate developmental or physiological responses in the immune system. The present invention provides a molecule which will be useful as a means to block the apoptosis signals, and variants of which will be useful in modulating signals leading to a plethora of immune conditions or responses.

SUMMARY OF THE INVENTION

The present invention is based, in part, upon the discovery of genes which encode proteins which exhibit sequence homology to receptors for TNF ligands. It provides various compositions and methods related to the UL144.

The present invention provides a substantially pure or recombinant polypeptide which: binds to a polyclonal antibody which binds to the mature SEQ ID NO: 2; and comprises at least two distinct segments of at least 8 contiguous amino acids of the mature SEQ ID NO: 2. Preferably, the segments include: a third distinct segment of at least 8 contiguous amino acids; at least one segment of at least 12 amino acids; or two of the segments of at least 10 amino acids each; or the polypeptide: in an in vitro Death Domain receptor assay, can block the response induced by its respective signaling ligand; or can modulate virus induced apoptosis. In other embodiments, the death domain receptor is selected from: Fas; TNF-R1; TRAMP; DR6; TRAIL-R1; TRAIL-R2; or TRAIL-R4. Preferably, the receptor assay is selected from: a cytotoxic death signal; an antiviral response; or induction of nitric

oxide synthase activity. In other embodiments, the polypeptide comprises a sequence of: mature SEQ ID NO: 2; residues 134-176 of SEQ ID NO: 2; LAWLSLFIFLVGIILLILYLIAA (residues 134-156 of SEQ ID NO: 2); YRSERC (residues 157-162 of SEQ ID NO: 2); RCQQCC (residues 161-166 of SEQ ID NO: 2); SIGKIFY (residues 167-173 of SEQ ID NO: 2); or KIFYRTL (residues 170-176 of SEQ ID NO: 2).

Nucleic acid embodiments of the present invention include a substantially pure or recombinant nucleic acid encoding the polypeptide described above. In various embodiments, such nucleic acid comprises: the mature polypeptide coding region of SEQ ID NO: 1; at least 17 contiguous nucleotides of the mature polypeptide coding portion of SEQ ID NO: 1; or at least 27 contiguous nucleotides of SEQ ID NO: 1. One preferred embodiment is an expression vector comprising sequence which hybridizes at 55 degrees centigrade and 400 mM salt to the mature polypeptide coding region of SEQ ID NO: 1, or which hybridizes at 65 degrees centigrade and/or 200 mM salt. Other embodiments include nucleic acids wherein the 17 contiguous nucleotides are from: nucleotides 400-468 of SEQ ID NO: 1; nucleotides 469-528 of SEQ ID NO: 1; or nucleotides 400-528 of SEQ ID NO: 1.

Methods provided include a method of modulating apoptosis of a cell, comprising exposing the cell to a sufficient amount of: a polypeptide described above, thereby protecting the cell; or a blocking compound which blocks the effect of UL144 on apoptosis. Typically, the apoptosis is mediated by a TNF-R family member; and may be induced by a TNF family member selected from TNF-α, FAS ligand, TRAIL, or TRAMP. In various embodiments, the modulating is reducing, and the polypeptide is administered in combination with: an immunostimulant; an antitumor reagent; or an antitumor treatment, including radiation therapy; or the modulating is increasing, the blocking compound is a monoclonal antibody or antisense polynucleotide, and the

blocking compound is administered in combination with: an antimicrobial agent; an immune adjuvant; or an antitumor treatment.

Various binding compounds are provided, e.g., a substantially pure binding compound comprising an antigen binding site from an antibody, wherein the antibody binds selectively to a polypeptide described above. Certain embodiments include the binding compound, wherein: the antibody is raised against a polypeptide described above; or the antibody is raised against a polypeptide comprising the mature SEQ ID NO: 2. Preferred embodiments include where the binding compound: is immunoselected; is a polyclonal antibody; binds to a denatured protein from SEQ ID NO: 2; exhibits a Kd to antigen of at least 30 mM; is attached to a solid substrate, including a bead or plastic membrane; is in a sterile composition; or is detectably labeled, including a radioactive or fluorescent label.

The invention also embraces various methods, including, e.g., a method of screening for a molecule which blocks a TNF family member induced apoptosis, the method comprising screening a compound library comprising a polypeptide described above for the ability to block the apoptosis. Another method is provided for screening for a molecule which interferes with UL144 anti-apoptotic activity, the method comprising screening a compound for its ability to block UL144 antiapoptotic activity. Preferably in such a method, the compound is selected from: an antibody which binds UL144; or an antisense nucleic acid comprising sequence complementary to a nucleic acid which encodes UL144.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

All references cited herein are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

I. General

Death receptors have been recently identified as a subgroup of the tumor necrosis factor (TNF) receptor superfamily with a predominant function in induction of apoptosis. See Tartaglia, et al. (1993) Cell 74:845-853; and Itoh and Nagata (1993) J. Biol. Chem. 268:10932-10937. The receptors are characterized by an intracellular region, called the death domain, which is required for the transmission of the cytotoxic signal. See Feinstein, et al. (1995) TIBS 20:342-344; Figure 6 of Itoh and Nagata (1993) J. Biol. Chem. 268:10932-10937; and Figure 3 of Tartaglia, et al. (1993) Cell 74:845-853. At least five different such death receptors are known, which include the CD95 (Fas/APO-1), the TNF receptor-1, TNF receptor apoptosis-mediated protein (TRAMP), Death receptor 6 (DR6), and TNF-related apoptosis-inducing ligand (TRAIL) receptor-1, -2, and -4. Other similar receptors have been identified with partial death domain motifs, e.g., the TNF-R2, which lacks a complete death domain, and TRAIL-R3, which is a decoy receptor.

The signaling pathways by which these receptors induce apoptosis are similar. Ligand binding induces receptor oligomerization, followed by the recruitment of an adapter protein to the death domain through homophillic interaction. The adapter protein then typically binds a proximal caspase, thereby connecting receptor signaling to the apoptotic effector machinery. Other mechanisms may include induction of nitric oxide (NO) synthase.

The host immune system of many organisms has several approaches of destroying infected cells. Lymphocytes can mediate cell-death via perforin and granzyme release, or alternatively, via the expression of FAS-Ligand, TRAIL, or TNF-α/β. However, suicide of viral infected cells would prevent further replication and viability of the infecting virus. Thus, a virus

would be conferred an adaptive advantage if it could block the apoptotic pathway. In fact, many viruses have developed an impressive array of molecules capable of blocking apoptotic cell-death pathways. Several Pox viruses express soluble TNF-R that antagonizes the effects of TNF-α; while adenovirus degrades FAS upon infection of cells. Several herpes viruses express proteins with death-effector domains, which can interfere with FLICE and FADD recruitment.

The human cytomegalovirus (HCMV) is a widespread infectious agent, and is a leading cause of congenital birth defects and mortality in the immunosuppressed. Herein is described the HCMV UL144, an ORF encoding a 176 aa transmembrane protein, which, based upon structural considerations, is closely related to members of the TNF-R family. The cytoplasmic tail of UL144 contains two motifs, one that is conserved in TNF-R1, FAS, and TRAMP; and the other that is present in the TRAIL receptors. Expression of UL144 leads to significant inhibition of cell-death induced by TNF-α, TRAIL, or FAS-L. In at least two HCMV strains, resistance to FAS-L and TRAIL induced apoptosis pathways correlated with UL144 expression. The presence of UL144 in the CMV strain is indicative of high virulence.

Antiviral activity would result from an antagonist which could block the viral blockage. The present invention provides means to block the viral mechanism of prevention of apoptosis. It also provides a useful method to screen for compounds which can block the viral interference. In these circumstances, the failure of the normal apoptotic mechanism may lead to a condition which results in negative organismal effects. Such an antiviral effect may be combined with other treatments which often accompany antiviral treatments, e.g., antimicrobial treatment, which may present opportunistic problems in a chronic or other viral infection.

In other contexts, overly efficient apoptosis may result in the downregulation or inefficient mounting of

an immune response. For example, in certain tumor situations, the efficient apoptosis of cells may prevent the immune system from mounting a more complete antitumor response. In such situations, the modification of the kinetics of the apoptosis may result in a more efficient antitumor response. Thus, a combination of other antitumor treatments with those provided herein may be utilized.

The present invention provides amino acid sequences and DNA sequences encoding various mammalian virus proteins, e.g., which are polypeptides derived from the UL144 open reading frame found in human cytomegalovirus (CMV). Among these proteins are those which modulate, e.g., prevent, apoptosis or programmed cell death. The full or partial length antigens, and fragments, or antagonists will be useful in physiological modulation of cells expressing receptors for, e.g., ligands of the TNF family.

The UL144 ORF has been hypothesized as a protein in human CMV. See Cha, et al. (1996) J. Virol. 70:78-83. However, no function or structural similarity to other genes was indicated in the GenBank report accession U33331. See also 1167929. A second GenBank report for AA262421 is indicated as "similar to ... ORF UL144". However, neither report seems to recognize any similarity to the TNF receptor family, or more specifically, the similarity to the death domain receptors.

Each of these proteins will also be useful as antigens, e.g., immunogens, for raising antibodies to various epitopes on the protein, linear and/or conformational epitopes. The molecules may be useful in defining various viral strains or subsets, either by the molecules produced by, or by expression of membrane forms of the receptors. As shown below, certain forms of the receptors serve as antagonists of the ligand, preventing mediation of the normal apoptotic signaling.

The UL144 ORF exhibits structural motifs characteristic of a member of the death domain receptor family. Table 1 provides the nucleic acid and predicted amino acid sequences for primate, e.g., human CMV UL144.

UL144 encodes a protein with a predicted 20 aa signal peptide and a 113 aa extracellular domain (ECD) that contains 7 putative N-linked and 3 putative O-linked glycosylation sites. The predicted size for the primary sequence is 20 kDa, but under reducing conditions it migrates at about 45 kDa. It is likely that the protein is highly glycosylated. Members of the TNF-R family contain cysteine-rich domains (CRD) in their extracellular regions, TNF-R1 contains four, FAS has three, and there are two in the TRAIL receptors. See Schulze-Osthoff, et al. (19998) Eur. J. Biochem. 254:439-459. The extracellular domains of UL144 align best, using the Clustal method, with domains 1 and 2 of TNF-R1. Based upon this structural analysis, UL144 with two CRD is a novel member of the TNF-R superfamily. UL144 has a predicted 24 residue transmembrane region (residues 134-156) and a 20 residue cytoplasmic tail. The UL144 cytoplasmic tail contains two notable motifs, a KxFxRxL sequence present in the death domains of TNF-R1, FAS, and TRAMP; and a second SxGKxxY motif present just distal to the TRAIL-R death domain. The FAS KxFxRxL motif resides within the α2 helix of its death-domain. This is the stretch responsible for homotypic aggregation of the FAS death-domains and the recruitment of FADD. Huang, et al. (1996) Nature 384:638-641. In addition, certain mutations within the KxFxRxL stretch in the TNF-R1 death-domain abrogate apoptosis signaling. Tartaglia, et al. (1993) Cell 74:845-853. Sequence information was obtained from GenBank and alignment performed using the Clustal method with Pam250 residue weight table (Megalign, DNASTAR software).

Table 1: CMV UL144 ORF nucleotide and polypeptide sequences SEQ ID NO: 1 and 2).

```
ATG AAG CCT CTG ATA ATG CTC ATC TGC TTT GCT GTG ATA TTA TTG CAG 48
Met Lys Pro Leu Ile Met Leu Ile Cys Phe Ala Val Ile Leu Leu Gln
 1               5                  10                  15

CTT GGA GTG ACT AAA GTG TGT CAG CAT AAT GAA GTG CAA CTG GGC AAT 96
Leu Gly Val Thr Lys Val Cys Gln His Asn Glu Val Gln Leu Gly Asn
            20                  25                  30

GAG TGC TGC CCT CCG TGT GGT TCG GGA CAA AGA GTT ACT AAA GTA TGC 144
Glu Cys Cys Pro Pro Cys Gly Ser Gly Gln Arg Val Thr Lys Val Cys
        35                  40                  45

ACG GAT TAT ACC AGT GTA ACG TGT ACC CCT TGC CCC AAC GGC ACG TAT 192
Thr Asp Tyr Thr Ser Val Thr Cys Thr Pro Cys Pro Asn Gly Thr Tyr
    50                  55                  60

GTA TCG GGA CTT TAC AAC TGT ACC GAT TGC ACT CAA TGT AAC GTC ACT 240
Val Ser Gly Leu Tyr Asn Cys Thr Asp Cys Thr Gln Cys Asn Val Thr
65                  70                  75                  80

CAG GTC ATG ATT CGT AAC TGC ACT TCC ACC AAT AAT ACC GTA TGC GCA 288
Gln Val Met Ile Arg Asn Cys Thr Ser Thr Asn Asn Thr Val Cys Ala
                85                  90                  95

CCT AAG AAC CAT ACG TAC TTT TCC ACT CCA GGC GTC CAA CAT CAC AAA 336
Pro Lys Asn His Thr Tyr Phe Ser Thr Pro Gly Val Gln His His Lys
            100                 105                 110

CAA CGA CAG CAA AAT CAT ACC GCA CAT ATA ACC GTC AAA CAA GGA AAA 384
Gln Arg Gln Gln Asn His Thr Ala His Ile Thr Val Lys Gln Gly Lys
        115                 120                 125

AGC GGT CGT CAT ACT CTA GCC TGG TTG TCT CTC TTT ATC TTT CTT GTG 432
Ser Gly Arg His Thr Leu Ala Trp Leu Ser Leu Phe Ile Phe Leu Val
    130                 135                 140

GGT ATC ATA CTT TTA ATT CTC TAT CTT ATA GCC GCC TAT CGG AGT GAG 480
Gly Ile Ile Leu Leu Ile Leu Tyr Leu Ile Ala Ala Tyr Arg Ser Glu
145                 150                 155                 160

AGA TGC CAA CAG TGT TGC TCA ATC GGC AAA ATT TTC TAC CGC ACC CTG 528
Arg Cys Gln Gln Cys Cys Ser Ile Gly Lys Ile Phe Tyr Arg Thr Leu
                165                 170                 175

TAA                                                         531

MKPLIMLICFAVILLQLGVTKVCQHNEVQLGNECCPPCGSGQRVTKVCTDYTSVTCTPCPNGT
YVSGLYNCTDCTQCNVTQVMIRNCTSTNNTVCAPKNHTYFSTPGVQHHKQRQQNHTAHITVKQ
GKSGRHTLAWLSLFIFLVGIILLILYLIAAYRSERCQQCCSIGKIFYRTL*
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Table 2: Alignment of related death domain receptor motifs of family members. Fas is SEQ ID NO: 3 (GenBank M67454); TNF-R1 is SEQ ID NO: 4 (SwissProt P19438); TRAMP is SEQ ID NO: 5 (GenBank AF026070); DR6 is SEQ ID NO: 6 (GenBank AF068868); TRAIL-R1 is SEQ ID NO: 7 (GenBank U90875); TRAIL-R2 is SEQ ID NO: 8 (GenBank AF016266); and TRAIL-R4 is SEQ ID NO: 9 (GenBank AF023849 or AF029761). Residue numbers are in parentheses.

...KIFYRTL... UL144 (170-176)
...KGFVRKN... Fas (246-252)
...KEFVRRL... TNF-R1 (372-378)
...KEFVRTL... TRAMP (357-363)
...KDIYQFL... DR6 (431-437)

...SIGKIFY... UL144 (167-173)
...DSGKFIY... TRAIL-R1 (450-456)
...SSGKFMY... TRAIL-R2 (424-428)
...GSEKLFY... TRAIL-R4 (368-374)

The natural antigens should be capable of modulating various biochemical responses which lead to biological or physiological responses in target cells, e.g., apoptosis. The embodiments characterized herein are from primate viruses, e.g., human, but other species variants almost surely exist, e.g., rodents, etc. The descriptions below are directed, for exemplary purposes, to primate CMV UL144, but are likewise applicable to related embodiments from other viruses or viruses of other species.

The UL144 ORF exhibits structural motifs characteristic of a member of the TNF receptor family. Compare, e.g., with the TNF receptor, NGF-receptor, and FAS receptor. Table 2 illustrates alignment of the predicted amino acid sequence to other forms of the TNF receptors from primate, e.g., human.

The structural homology of these genes to the TNF ligand receptor family suggests related function of these molecules. Receptor family antagonists may act as blockers of a co-stimulatory interaction needed for, e.g., T cell mediated cell activation.

TNF ligand molecules typically modulate cell survival, proliferation, viability, and/or differentiation. For example, TNF and FAS can kill cells expressing their respective receptors, including fibroblasts, liver cells, and lymphocytes. Some members

of this class of ligands exhibit effects on cellular proliferation of cells expressing their respective receptors, e.g., B cells expressing CD40. These effects on apoptosis or proliferation may also effect subsequent differentiation steps, and may lead, directly or indirectly, to changes in cytokine expression profiles.

The members of the TNF ligand family also exhibit costimulation effects, which may also regulate cellular differentiation or apoptosis. Receptor expressing cells may be protected from activation induced cell death (AICD) or apoptosis. For example, CD40 ligand can have effects on T and B lymphocytes.

The embodiments characterized herein are from human viruses, but additional sequences for proteins in other mammalian species, e.g., primates and rodents, will also be available. See below.

II. Purified Receptor

Human UL144 amino acid sequence is shown in SEQ ID NO: 2. The amino acid sequence, provided amino to carboxy, is important in providing sequence information on the antigen allowing for distinguishing the protein from other proteins and exemplifying numerous variants. Moreover, the amino acid sequences allow preparation of polypeptides to generate antibodies to recognize such segments, and nucleotide sequences allow preparation of oligonucleotide probes, both of which are strategies for detection or isolation, e.g., cloning, of genes or cDNAs encoding such sequences.

As used herein, the term "human CMV UL144" shall encompass, when used in a protein context, a protein having amino acid sequence shown in SEQ ID NO: 2. Significant fragments of such a protein should preserve at least some of the properties of the full length mature protein, e.g., immunogenicity or antigenicity. Other essentially identical proteins may be found in other primate viruses, including strain differences. In addition, binding components, e.g., antibodies,

typically bind to a UL144 with high affinity, e.g., at least about 100 nM, usually better than about 30 nM, preferably better than about 10 nM, and more preferably at better than about 3 nM. Homologous proteins would be found in other mammalian viruses, e.g., primates or rodents, including variant strains. Non-mammalian species should also possess structurally or functionally related genes and proteins, e.g., birds or amphibians.

The term "polypeptide" as used herein includes a significant fragment or segment, and encompasses a stretch of amino acid residues of at least about 8 amino acids, generally at least about 12 amino acids, typically at least about 16 amino acids, preferably at least about 20 amino acids, and, in particularly preferred embodiments, at least about 30 or more amino acids, e.g., 35, 40, 45, 50, 70, 90, and more. In certain embodiments, there will be a plurality of distinct, e.g., nonoverlapping, segments of the specified length. Typically, the plurality will be at least two, more usually at least three, and preferably 5, 7, or even more. While the length minima are provided, longer lengths, of various sizes, may be appropriate, e.g., one of length 7, and two of length 12.

The term "binding composition" refers to molecules that bind with specificity to the receptor, e.g., UL144, e.g., in a cell adhesion pairing type fashion, or an antibody-antigen interaction. Other compounds include, e.g., proteins, which specifically associate with UL144, including in a natural physiologically relevant protein-protein interaction, either covalent or non-covalent. The molecule may be a polymer, or chemical reagent. A functional analog may be an antigen with structural modifications, or it may be a molecule which has a molecular shape which interacts with the appropriate binding determinants. The compounds may serve as agonists or antagonists of the binding interaction, see, e.g., Goodman, et al. (eds. 1996) Goodman & Gilman's:

The Pharmacological Bases of Therapeutics (9th ed.), McGraw-Hill.

Substantially pure typically means that the protein is free from other contaminating proteins, nucleic acids, or other biologicals derived from the original source organism. Purity may be assayed by standard methods, typically by weight, and will ordinarily be at least about 40% pure, generally at least about 50% pure, often at least about 60% pure, typically at least about 80% pure, preferably at least about 90% pure, and in most preferred embodiments, at least about 95% pure. Carriers or excipients will often be added.

Solubility of a polypeptide or fragment depends upon the environment and the polypeptide. Many parameters affect polypeptide solubility, including temperature, electrolyte environment, size and molecular characteristics of the polypeptide, and nature of the solvent. Typically, the temperature at which the polypeptide is used ranges from about 4° C to about 65° C. Usually the temperature at use is greater than about 18° C. For diagnostic purposes, the temperature will usually be about room temperature or warmer, but less than the denaturation temperature of components in the assay. For therapeutic purposes, the temperature will usually be body temperature, typically about 37° C for humans and mice, though under certain situations the temperature may be raised or lowered in situ or in vitro.

The size and structure of the polypeptide should generally be in a substantially stable state, and usually not in a denatured state. The polypeptide may be associated with other polypeptides in a quaternary structure, e.g., to confer solubility, or associated with lipids or detergents in a manner which approximates natural lipid bilayer interactions. In other contexts, denatured antigen may be useful, e.g., in diagnostic applications.

The solvent and electrolytes will usually be a biologically compatible buffer, of a type used for preservation of biological activities, and will usually approximate a physiological aqueous solvent. Usually the solvent will have a neutral pH, typically between about 5 and 10, and preferably about 7.5. On some occasions, one or more detergents will be added, typically a mild non-denaturing one, e.g., CHS (cholesteryl hemisuccinate) or CHAPS (3-[3-cholamidopropyl)dimethylammonio]-1-propane sulfonate), or a low enough concentration as to avoid significant disruption of structural or physiological properties of the protein.

III. Physical Variants

This invention also encompasses proteins or peptides having substantial amino acid sequence identity with the amino acid sequence of the UL144 receptor. The variants include strain, species, polymorphic, or allelic variants, often having a sequence different from natural forms.

Amino acid sequence homology, or sequence identity, is determined by optimizing residue matches, if necessary, by introducing gaps as required. See also Needleham, et al. (1970) J. Mol. Biol. 48:443-453; Sankoff, et al. (1983) Chapter One in Time Warps, String Edits, and Macromolecules: The Theory and Practice of Sequence Comparison, Addison-Wesley, Reading, MA; and software packages from NCBI, National Institutes of Health; and the University of Wisconsin Genetics Computer Group, Madison, WI. Sequence identity changes when considering conservative substitutions as matches. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. Homologous amino acid sequences are typically intended

to include natural polymorphic or allelic and interspecies variations in each respective protein sequence. Typical homologous proteins or peptides will have from 25-100% identity (if gaps can be introduced), to 50-100% identity (if conservative substitutions are included) with the amino acid sequence of the UL144. Identity measures will be at least about 35%, generally at least about 40%, often at least about 50%, typically at least about 60%, usually at least about 70%, preferably at least about 80%, and more preferably at least about 90%.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Optical alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2:482, by the homology alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson and Lipman (1988) Proc. Nat'l Acad. Sci. USA 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (see generally Ausubel et al., supra).

One example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments to show relationship and percent sequence identity. It also plots a tree or dendrogram showing

the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng and Doolittle (1987) J. Mol. Evol. 35:351-360. The method used is similar to the method described by Higgins and Sharp (1989) CABIOS 5:151-153. The program can align up to 300 sequences, each of a maximum length of 5,000 nucleotides or amino acids. The multiple alignment procedure begins with the pairwise alignment of the two most similar sequences, producing a cluster of two aligned sequences. This cluster is then aligned to the next most related sequence or cluster of aligned sequences. Two clusters of sequences are aligned by a simple extension of the pairwise alignment of two individual sequences. The final alignment is achieved by a series of progressive, pairwise alignments. The program is run by designating specific sequences and their amino acid or nucleotide coordinates for regions of sequence comparison and by designating the program parameters. For example, a reference sequence can be compared to other test sequences to determine the percent sequence identity relationship using the following parameters: default gap weight (3.00), default gap length weight (0.10), and weighted end gaps.

Another example of algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described Altschul, et al. (1990) J. Mol. Biol. 215:403-410. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http:www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul, et al., supra). These

initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a wordlength (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1989) Proc. Nat'l Acad. Sci. USA 89:10915) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin and Altschul (1993) Proc. Nat'l Acad. Sci. USA 90:5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

A further indication that two nucleic acid sequences of polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical

to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions, as described below.

The isolated UL144 DNA can be readily modified by nucleotide substitutions, nucleotide deletions, nucleotide insertions, and inversions of nucleotide stretches. These modifications result in novel DNA sequences which encode these antigens, their derivatives, or proteins having similar physiological, immunogenic, antigenic, or other functional activity. These modified sequences can be used to produce mutant antigens or to enhance expression. Enhanced expression may involve gene amplification, increased transcription, increased translation, and other mechanisms. "Mutant UL144" encompasses a polypeptide otherwise falling within the sequence identity definition of the UL144 ORF as set forth above, but having an amino acid sequence which differs from that of UL144 as normally found in nature, whether by way of deletion, substitution, or insertion. This generally includes proteins having significant identity with a protein having a sequence of SEQ ID NO: 2, and as sharing various biological activities, e.g., antigenic or immunogenic, with those sequences, and in preferred embodiments contain most of the full length disclosed sequences. Full length sequences will typically be preferred, though truncated versions, e.g., soluble constructs and intact domains, will also be useful, likewise, genes or proteins found from natural sources are typically most desired. Similar concepts apply to different UL144 proteins, particularly those found in viruses from various warm blooded animals, e.g., mammals and birds. These descriptions are generally meant to encompass a variety of UL144 proteins, not limited to the particular human embodiment specifically discussed.

UL144 mutagenesis can also be conducted by making amino acid insertions or deletions. Substitutions, deletions, insertions, or any combinations may be generated to arrive at a final construct. Insertions include amino- or carboxy- terminal fusions. Random mutagenesis can be conducted at a target codon and the expressed mutants can then be screened for the desired activity. Methods for making substitution mutations at predetermined sites in DNA having a known sequence are well known in the art, e.g., by M13 primer mutagenesis or polymerase chain reaction (PCR) techniques. See, e.g., Sambrook, et al. (1989); Ausubel, et al. (1987 and Supplements); and Kunkel, et al. (1987) Methods in Enzymol. 154:367-382.

The present invention also provides recombinant proteins, e.g., heterologous fusion proteins using segments from these proteins. A heterologous fusion protein is a fusion of proteins or segments which are naturally not normally fused in the same manner. A similar concept applies to heterologous nucleic acid sequences. Fusion proteins will be useful as sources for cleaving, separating, and purifying portions thereof.

In addition, new constructs may be made from combining similar functional domains from other proteins. For example, target-binding or other segments may be "swapped" between different new fusion polypeptides or fragments. See, e.g., Cunningham, et al. (1989) Science 243:1330-1336; and O'Dowd, et al. (1988) J. Biol. Chem. 263:15985-15992. In particular, the intracellular portions of the UL144 may be fused to transmembrane segments.

The phosphoramidite method described by Beaucage and Carruthers (1981) Tetra. Letts. 22:1859-1862, will produce suitable synthetic DNA fragments. A double stranded fragment will often be obtained either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by

adding the complementary strand using DNA polymerase with an appropriate primer sequence, e.g., PCR techniques. Various complementary, or antisense, nucleic acids are also provided, and may be used as translation blocking reagents.

IV. Functional Variants

The blocking of physiological response with UL144 may result from the inhibition of binding of the respective ligand to signaling form of receptor, e.g., transmembrane form of receptor, likely through competitive inhibition. However, certain data suggest that the mechanism may be blocking of recruitment of functional receptor complexes. Thus, in vitro assays of the present invention will often use isolated protein, soluble fragments comprising ligand binding segments of these proteins, or forms attached to solid phase substrates. Certain expression constructs may be created which produce receptor complexes which may be targets to block the interaction of the UL144 with other components of death domain receptors. These assays will also allow for the diagnostic determination of the effects of various mutations and modifications, e.g., UL144 analogs. In addition, the assays will allow for screening of compounds which disrupt the formation of complexes.

This invention also contemplates the use of competitive drug screening assays, e.g., where neutralizing antibodies to antigen or binding fragments compete with a test compound for binding to the protein, e.g., of natural protein sequence. Other screening assays allow for identification of compounds which block the formation of nonfunctional apoptotic receptor complexes.

"Derivatives" of receptor antigens include amino acid sequence mutants from naturally occurring forms, glycosylation variants, and covalent or aggregate conjugates with other chemical moieties. Covalent

derivatives can be prepared by linkage of functionalities to groups which are found in receptor amino acid side chains or at the N- or C- termini, e.g., by standard means. See, e.g., Lundblad and Noyes (1988) Chemical Reagents for Protein Modification, vols. 1-2, CRC Press, Inc., Boca Raton, FL; Hugli (ed. 1989) Techniques in Protein Chemistry, Academic Press, San Diego, CA; and Wong (1991) Chemistry of Protein Conjugation and Cross Linking, CRC Press, Boca Raton, FL.

In particular, glycosylation alterations are included, e.g., made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing, or in further processing steps. See, e.g., Elbein (1987) Ann. Rev. Biochem. 56:497-534. Also embraced are versions of the peptides with the same primary amino acid sequence which have other minor modifications, including phosphorylated amino acid residues, e.g., phosphotyrosine, phosphoserine, or phosphothreonine.

Fusion polypeptides between UL144 and other homologous or heterologous proteins are also provided. Many cytokine receptors or other surface proteins are multimeric, e.g., homodimeric entities, and a repeat construct may have various advantages, including lessened susceptibility to proteolytic cleavage. Typical examples are fusions of an epitope tag or reporter polypeptide, e.g., luciferase, with a segment or domain of a protein, e.g., a receptor-binding segment, so that the presence or location of the fused ligand may be easily determined. See, e.g., Dull, et al., U.S. Patent No. 4,859,609. Other gene fusion partners include bacterial ß-galactosidase, trpE, Protein A, ß-lactamase, alpha amylase, alcohol dehydrogenase, yeast alpha mating factor, and detection or purification tags such as a FLAG sequence of His6 sequence. See, e.g., Godowski, et al. (1988) Science 241:812-816. Of particular interest are fusion

constructs of the receptor with a membrane attachment domain.

Fusion peptides will typically be made by either recombinant nucleic acid methods or by synthetic polypeptide methods. Techniques for nucleic acid manipulation and expression are described generally, e.g., in Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual (2d ed.), vols. 1-3, Cold Spring Harbor Laboratory; and Ausubel, et al. (eds. 1993) Current Protocols in Molecular Biology, Greene and Wiley, NY. Techniques for synthesis of polypeptides are described, e.g., in Merrifield (1963) J. Amer. Chem. Soc. 85:2149-2156; Merrifield (1986) Science 232: 341-347; Atherton, et al. (1989) Solid Phase Peptide Synthesis: A Practical Approach, IRL Press, Oxford; and Grant (1992) Synthetic Peptides: A User's Guide, W.H. Freeman, NY.

This invention also contemplates the use of derivatives of UL144 other than variations in amino acid sequence or glycosylation. Such derivatives may involve covalent or aggregative association with chemical moieties. Covalent or aggregative derivatives will be useful as immunogens, as reagents in immunoassays, or in purification methods such as for affinity purification of binding partners, e.g., other antigens. A UL144 can be immobilized by covalent bonding to a solid support such as cyanogen bromide-activated SEPHAROSE, by methods which are well known in the art, or adsorbed onto polyolefin surfaces, with or without glutaraldehyde cross-linking, for use in the assay or purification of antibodies or an alternative binding composition. The UL144 can also be labeled with a detectable group, e.g., for use in diagnostic assays. Purification of receptor may be effected by an immobilized antibody or complementary binding partner.

A solubilized receptor or fragment of this invention can be used as an immunogen for the production of antisera or antibodies specific for binding to the

antigen or fragments thereof. Purified antigen can be used to screen monoclonal antibodies or antigen-binding fragments, encompassing antigen binding fragments of natural antibodies, e.g., Fab, Fab', $F(ab)_2$, etc. Purified UL144 can also be used as a reagent to detect antibodies generated in response to the presence of elevated levels of the antigen or cell fragments containing the antigen, both of which may be diagnostic of an abnormal or specific physiological or disease condition. This invention contemplates antibodies raised against amino acid sequences encoded by nucleotide sequence shown in SEQ ID NO: 1, or fragments of proteins containing it. In particular, this invention contemplates antibodies having binding affinity to or being raised against specific fragments which are predicted to lie outside of the lipid bilayer, both extracellular or intracellular.

The present invention contemplates the isolation of additional closely related strains or virus species variants. Southern and Northern blot analysis should establish that similar genetic entities exist in other mammals, and may well be useful in diagnosing the virulence of different CMV strains. It is likely that these receptors are widespread in virus species variants, e.g., in rodents, lagomorphs, carnivores, artiodactyla, perissodactyla, and primates.

The invention also provides means to isolate a group of related antigens displaying both distinctness and similarities in structure, expression, and function. Elucidation of many of the physiological effects of the molecules will be greatly accelerated by the isolation and characterization of additional distinct species variants of them. In particular, the present invention provides useful probes for identifying additional homologous genetic entities in different strains or viruses of other species.

The isolated genes will allow transformation of cells lacking expression of a corresponding receptor,

e.g., either species types or cells which lack corresponding antigens and exhibit negative background activity. This should allow analysis of the function of receptor in comparison to untransformed control cells.

Dissection of critical structural elements which effect the various activation or differentiation functions mediated through these antigens is possible using standard techniques of modern molecular biology, particularly in comparing members of the related class. See, e.g., the homolog-scanning mutagenesis technique described in Cunningham, et al. (1989) Science 243:1339-1336; and approaches used in O'Dowd, et al. (1988) J. Biol. Chem. 263:15985-15992; and Lechleiter, et al. (1990) EMBO J. 9:4381-4390.

Intracellular functions would probably involve segments of the antigen which are normally accessible to the cytosol of transmembrane forms of the receptors. However, protein internalization may occur under certain circumstances, and interaction between intracellular components and "extracellular" segments may occur. The specific segments of interaction of receptor with other intracellular components may be identified by mutagenesis or direct biochemical means, e.g., cross-linking or affinity methods. Structural analysis by crystallographic or other physical methods will also be applicable. Further investigation of the mechanism of signal transduction will include study of associated components which may be isolatable by affinity methods or by genetic means, e.g., complementation analysis of mutants.

Further study of the expression and control of UL144 will be pursued. The controlling elements associated with the antigen should exhibit differential physiological, developmental, tissue specific, or other expression patterns. Mechanisms to block expression may be useful for blocking virulence of UL144 containing CMV strains. Upstream or downstream genetic regions, e.g., control elements, are of interest. In particular,

physiological or developmental variants, e.g., multiple alternatively processed forms of the antigen might be found. See, e.g., SEQ ID NO: 1. Thus, differential splicing of message may lead to an assortment of membrane bound forms, soluble forms, and modified versions of antigen.

Structural studies of the antigens will lead to design of new antigens, particularly analogs exhibiting agonist or antagonist properties on the molecule. This can be combined with previously described screening methods to isolate antigens exhibiting desired spectra of activities.

V. Antibodies

Antibodies can be raised to various receptors, including species, polymorphic, or allelic variants, and fragments thereof, both in their naturally occurring forms and in their recombinant forms. Additionally, antibodies can be raised to UL144 ORF in either its active forms or in its inactive forms, including native or denatured versions. Anti-idiotypic antibodies are also contemplated.

Antibodies, including binding fragments and single chain versions, against predetermined fragments of the antigens can be raised by immunization of animals with conjugates of the fragments with immunogenic proteins. Monoclonal antibodies are prepared from cells secreting the desired antibody. These antibodies can be screened for binding to normal or defective UL144, or screened for agonistic or antagonistic activity, e.g., mediated through the antigen or its binding partner. Antibodies may be agonistic or antagonistic, e.g., by sterically blocking ligand binding. These monoclonal antibodies will usually bind with at least a K_D of about 1 mM, more usually at least about 300 μM, typically at least about 100 μM, more typically at least about 30 μM, preferably at least about 10 μM, and more preferably at least about 3 μM or better.

The antibodies of this invention can also be useful in diagnostic applications. As capture or non-neutralizing antibodies, they can be screened for ability to bind to the antigens without inhibiting binding by a partner. As neutralizing antibodies, they can be useful in competitive binding assays. They will also be useful in detecting or quantifying UL144 protein or its binding partners. See, e.g., Chan (ed. 1987) Immunology: A Practical Guide, Academic Press, Orlando, FL; Price and Newman (eds. 1991) Principles and Practice of Immunoassay, Stockton Press, N.Y.; and Ngo (ed. 1988) Nonisotopic Immunoassay, Plenum Press, N.Y. Cross absorptions or other tests will identify antibodies which exhibit various spectra of specificities, e.g., unique or shared species specificities. Such diagnostic assays may identify particularly virulent or non-virulent CMV strains.

Further, the antibodies, including antigen binding fragments, of this invention can be potent antagonists that bind to the antigen and inhibit functional binding or inhibit the ability of a binding partner to elicit a biological response. They also can be useful as non-neutralizing antibodies and can be coupled to toxins or radionuclides so that when the antibody binds to antigen, a cell expressing it, e.g., on its surface, is killed. Further, these antibodies can be conjugated to drugs or other therapeutic agents, either directly or indirectly by means of a linker, and may effect drug targeting.

Antigen fragments may be joined to other materials, particularly polypeptides, as fused or covalently joined polypeptides to be used as immunogens. An antigen and its fragments may be fused or covalently linked to a variety of immunogens, such as keyhole limpet hemocyanin, bovine serum albumin, tetanus toxoid, etc. See Microbiology, Hoeber Medical Division, Harper and Row, 1969; Landsteiner (1962) Specificity of Serological Reactions, Dover Publications, New York; Williams, et

al. (1967) Methods in Immunology and Immunochemistry, vol. 1, Academic Press, New York; and Harlow and Lane (1988) Antibodies: A Laboratory Manual, CSH Press, NY, for descriptions of methods of preparing polyclonal antisera.

In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts, such as mice, rodents, primates, humans, etc. Description of techniques for preparing such monoclonal antibodies may be found in, e.g., Stites, et al. (eds.) Basic and Clinical Immunology (4th ed.), Lange Medical Publications, Los Altos, CA, and references cited therein; Harlow and Lane (1988) Antibodies: A Laboratory Manual, CSH Press; Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed.), Academic Press, New York; and particularly in Kohler and Milstein (1975) in Nature 256:495-497, which discusses one method of generating monoclonal antibodies.

Other suitable techniques involve in vitro exposure of lymphocytes to the antigenic polypeptides or alternatively to selection of libraries of antibodies in phage or similar vectors. See, Huse, et al. (1989) "Generation of a Large Combinatorial Library of the Immunoglobulin Repertoire in Phage Lambda," Science 246:1275-1281; and Ward, et al. (1989) Nature 341:544-546. The polypeptides and antibodies of the present invention may be used with or without modification, including chimeric or humanized antibodies. Frequently, the polypeptides and antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like. Patents, teaching the use of such labels include U.S. Patent Nos.

3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant immunoglobulins may be produced, see Cabilly, U.S. Patent No. 4,816,567; Moore, et al., U.S. Patent No. 4,642,334; and Queen, et al. (1989) Proc. Nat'l Acad. Sci. USA 86:10029-10033.

The antibodies of this invention can also be used for affinity chromatography in isolating the protein. Columns can be prepared where the antibodies are linked to a solid support. See, e.g., Wilchek, et al. (1984) Meth. Enzymol. 104:3-55.

Antibodies raised against UL144 will also be useful to raise anti-idiotypic antibodies. These will be useful in detecting or diagnosing various immunological conditions related to expression of the respective antigens.

VI. Nucleic Acids

The described peptide sequences and the related reagents are useful in detecting, isolating, or identifying a DNA clone encoding UL144, e.g., from a natural source. Typically, it will be useful in isolating a gene from mammal, and similar procedures will be applied to isolate genes from other species, e.g., warm blooded animals, such as birds and mammals. Cross hybridization will allow isolation of UL144 from other strains of viruses, or species variant viruses. A number of different approaches should be available to successfully isolate a suitable nucleic acid clone.

The purified protein or defined peptides are useful for generating antibodies by standard methods, as described above. Synthetic peptides or purified protein can be presented to an immune system to generate monoclonal or polyclonal antibodies. See, e.g., Coligan (1991) Current Protocols in Immunology Wiley/Greene; and Harlow and Lane (1989) Antibodies: A Laboratory Manual, Cold Spring Harbor Press. Alternatively, the UL144 can be used as a specific binding reagent, and advantage can

be taken of its specificity of binding, much like an antibody would be used.

For example, the specific binding composition could be used for screening of an expression library made from a cell line which expresses a UL144 polypeptide. The screening can be standard staining of surface expressed antigen constructs, or by panning. Screening of intracellular expression can also be performed by various staining or immunofluorescence procedures. The binding compositions could be used to affinity purify or sort out cells expressing the protein.

The peptide segments can also be used to predict appropriate oligonucleotides to screen a library. The genetic code can be used to select appropriate oligonucleotides useful as probes for screening. See, e.g., SEQ ID NO: 1. In combination with polymerase chain reaction (PCR) techniques, synthetic oligonucleotides will be useful in selecting correct clones from a library. Complementary sequences will also be used as probes, primers, or antisense strands. Based upon identification of the likely extracellular domain, various fragments should be particularly useful, e.g., coupled with anchored vector or poly-A complementary PCR techniques or with complementary DNA of other peptides.

This invention contemplates use of isolated DNA or fragments to encode a biologically active corresponding UL144 polypeptide. In addition, this invention covers isolated or recombinant DNA which encodes a biologically active protein or polypeptide which is capable of hybridizing under appropriate conditions with the DNA sequences described herein. Said biologically active protein or polypeptide can be an intact antigen, or fragment, and have an amino acid sequence disclosed in, e.g., SEQ ID NO: 2. Further, this invention covers the use of isolated or recombinant DNA, or fragments thereof, which encode proteins which are homologous to a receptor or which was isolated using cDNA encoding a

receptor as a probe. The isolated DNA can have the respective regulatory sequences in the 5' and 3' flanks, e.g., promoters, enhancers, poly-A addition signals, and others.

An "isolated" nucleic acid is a nucleic acid, e.g., an RNA, DNA, or a mixed polymer, which is substantially separated from other components which naturally accompany a native sequence, e.g., ribosomes, polymerases, and/or flanking genomic sequences from the originating species. The term embraces a nucleic acid sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogs or analogs biologically synthesized by heterologous systems. A substantially pure molecule includes isolated forms of the molecule. Generally, the nucleic acid will be in a vector or fragment less than about 50 kb, usually less than about 30 kb, typically less than about 10 kb, and preferably less than about 6 kb.

An isolated nucleic acid will generally be a homogeneous composition of molecules, but will, in some embodiments, contain minor heterogeneity. This heterogeneity is typically found at the polymer ends or portions not critical to a desired biological function or activity.

A "recombinant" nucleic acid is defined either by its method of production or its structure. In reference to its method of production, e.g., a product made by a process, the process is use of recombinant nucleic acid techniques, e.g., involving human intervention in the nucleotide sequence, typically selection or production. Alternatively, it can be a nucleic acid made by generating a sequence comprising fusion of two fragments which are not naturally contiguous to each other, but is meant to exclude products of nature, e.g., naturally occurring mutants. Thus, e.g., products made by transforming cells with any unnaturally occurring vector is encompassed, as are nucleic acids comprising sequence

derived using any synthetic oligonucleotide process. Such is often done to replace a codon with a redundant codon encoding the same or a conservative amino acid, while typically introducing or removing a sequence recognition site.

Alternatively, it is performed to join together nucleic acid segments of desired functions to generate a single genetic entity comprising a desired combination of functions not found in the commonly available natural forms. Restriction enzyme recognition sites are often the target of such artificial manipulations, but other site specific targets, e.g., promoters, DNA replication sites, regulation sequences, control sequences, or other useful features may be incorporated by design. A similar concept is intended for a recombinant, e.g., fusion, polypeptide. Specifically included are synthetic nucleic acids which, by genetic code redundancy, encode polypeptides similar to fragments of these antigens, and fusions of sequences from various different species variants.

A significant "fragment" in a nucleic acid context is a contiguous segment of at least about 17 nucleotides, generally at least about 22 nucleotides, ordinarily at least about 29 nucleotides, more often at least about 35 nucleotides, typically at least about 41 nucleotides, usually at least about 47 nucleotides, preferably at least about 55 nucleotides, and in particularly preferred embodiments will be at least about 60 or more nucleotides.

A DNA which codes for a UL144 polypeptide will be particularly useful to identify genes, mRNA, and cDNA species which code for related or homologous proteins, as well as DNAs which code for homologous proteins from different species. There are likely homologs in other species, including primates, rodents, and birds. Various receptor proteins should be homologous and are encompassed herein. However, even genes encoding proteins that have a more distant evolutionary

relationship to the antigen can readily be isolated under appropriate conditions using these sequences if they are sufficiently homologous. Primate viruses which express UL144 proteins are of particular interest.

Recombinant clones derived from the genomic sequences, e.g., containing introns, will be useful for transgenic studies, including, e.g., transgenic cells and organisms, and for gene therapy. See, e.g., Goodnow (1992) "Transgenic Animals" in Roitt (ed.) Encyclopedia of Immunology, Academic Press, San Diego, pp. 1502-1504; Travis (1992) Science 256:1392-1394; Kuhn, et al. (1991) Science 254:707-710; Capecchi (1989) Science 244:1288; Robertson (1987)(ed.) Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, IRL Press, Oxford; and Rosenberg (1992) J. Clinical Oncology 10:180-199.

Substantial homology in the nucleic acid sequence comparison context means either that the segments, or their complementary strands, when compared, are identical when optimally aligned, with appropriate nucleotide insertions or deletions, in at least about 50% of the nucleotides, generally at least about 58%, ordinarily at least about 65%, often at least about 71%, typically at least about 77%, usually at least about 85%, preferably at least about 95 to 98% or more, and in particular embodiments, as high as about 99% or more of the nucleotides. Alternatively, substantial homology exists when the segments will hybridize under selective hybridization conditions, to a strand, or its complement, typically using a sequence of UL144, e.g., in SEQ ID NO: 1. Typically, selective hybridization will occur when there is at least about 55% homology over a stretch of at least about 30 nucleotides, preferably at least about 75% over a stretch of about 25 nucleotides, and most preferably at least about 90% over about 20 nucleotides. See, Kanehisa (1984) Nuc. Acids Res. 12:203-213. The length of homology comparison, as described, may be over longer stretches, and in certain embodiments will be over a stretch of at least about 17

nucleotides, usually at least about 28 nucleotides, typically at least about 40 nucleotides, and preferably at least about 75 to 100 or more nucleotides.

Stringent conditions, in referring to homology in the hybridization context, will be stringent combined conditions of salt, temperature, organic solvents, and other parameters, typically those controlled in hybridization reactions. Stringent temperature conditions will usually include temperatures in excess of about 30° C, usually in excess of about 37° C, typically in excess of about 55° C, preferably in excess of about 70° C. Stringent salt conditions will ordinarily be less than about 1000 mM, usually less than about 400 mM, typically less than about 250 mM, preferably less than about 150 mM. However, the combination of parameters is much more important than the measure of any single parameter. See, e.g., Wetmur and Davidson (1968) J. Mol. Biol. 31:349-370. Hybridization under stringent conditions should give a background of at least 2-fold over background, preferably at least 3-5 or more.

UL144 from other mammalian species can be cloned and isolated by cross-species hybridization of closely related species. Homology may be relatively low between distantly related species, and thus hybridization of relatively closely related species is advisable. Alternatively, preparation of an antibody preparation which exhibits less species specificity may be useful in expression cloning approaches.

VII. Making Receptors; Mimetics

DNA which encodes the UL144 or fragments thereof can be obtained by chemical synthesis, screening cDNA libraries, or screening genomic libraries prepared from a wide variety of cell lines or tissue samples. See, e.g., Okayama and Berg (1982) Mol. Cell. Biol. 2:161-170; Gubler and Hoffman (1983) Gene 25:263-269; and Glover (ed. 1984) DNA Cloning: A Practical Approach,

IRL Press, Oxford. Alternatively, the sequences provided herein provide useful PCR primers or allow synthetic or other preparation of suitable genes encoding a receptor; including, naturally occurring embodiments.

This DNA can be expressed in a wide variety of host cells for the synthesis of a full-length UL144 or fragments which can in turn, e.g., be used to generate polyclonal or monoclonal antibodies; for binding studies; for construction and expression of modified molecules; and for structure/function studies.

Vectors, as used herein, comprise plasmids, viruses, bacteriophage, integratable DNA fragments, and other vehicles which enable the integration of DNA fragments into the genome of the host. See, e.g., Pouwels, et al. (1985 and Supplements) Cloning Vectors: A Laboratory Manual, Elsevier, N.Y.; and Rodriguez, et al. (1988 eds.) Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Buttersworth, Boston, MA.

For purposes of this invention, DNA sequences are operably linked when they are functionally related to each other. For example, DNA for a presequence or secretory leader is operably linked to a polypeptide if it is expressed as a preprotein or participates in directing the polypeptide to the cell membrane or in secretion of the polypeptide. A promoter is operably linked to a coding sequence if it controls the transcription of the polypeptide; a ribosome binding site is operably linked to a coding sequence if it is positioned to permit translation. Usually, operably linked means contiguous and in reading frame, however, certain genetic elements such as repressor genes are not contiguously linked but still bind to operator sequences that in turn control expression. See e.g., Rodriguez, et al., Chapter 10, pp. 205-236; Balbas and Bolivar (1990) Methods in Enzymol. 185:14-37; and Ausubel, et al. (1993) Current Protocols in Molecular Biology, Greene and Wiley, NY.

Representative examples of suitable expression vectors include pCDNA1; pCD, see Okayama, et al. (1985) Mol. Cell Biol. 5:1136-1142; pMC1neo Poly-A, see Thomas, et al. (1987) Cell 51:503-512; and a baculovirus vector such as pAC 373 or pAC 610. See, e.g., Miller (1988) Ann. Rev. Microbiol. 42:177-199.

It will often be desired to express an UL144 polypeptide in a system which provides a specific or defined glycosylation pattern. See, e.g., Luckow and Summers (1988) Bio/Technology 6:47-55; and Kaufman (1990) Meth. Enzymol. 185:487-511. Preferred prokaryotic forms lack eukaryotic glycosylation patterns.

The UL144, or a fragment thereof, may be engineered to be phosphatidyl inositol (PI) linked to a cell membrane, but can be removed from membranes by treatment with a phosphatidyl inositol cleaving enzyme, e.g., phosphatidyl inositol phospholipase-C. This releases the antigen in a biologically active form, and allows purification by standard procedures of protein chemistry. See, e.g., Low (1989) Biochim. Biophys. Acta 988:427-454; Tse, et al. (1985) Science 230:1003-1008; and Brunner, et al. (1991) J. Cell Biol. 114:1275-1283.

Now that the UL144 has been characterized, fragments or derivatives thereof can be prepared by conventional processes for synthesizing peptides. These include processes such as are described in Stewart and Young (1984) Solid Phase Peptide Synthesis, Pierce Chemical Co., Rockford, IL; Bodanszky and Bodanszky (1984) The Practice of Peptide Synthesis, Springer-Verlag, New York, NY; Bodanszky (1984) The Principles of Peptide Synthesis, Springer-Verlag, New York; and Villafranca (ed. 1991) Techniques in Protein Chemistry II, Academic Press, San Diego, Ca.

VIII. Uses

The present invention provides reagents which will find use in diagnostic applications as described

elsewhere herein, e.g., in the general description for T cell mediated conditions, or below in the description of kits for diagnosis. The genes will be useful in forensic analyses, e.g., to identify virulence, to identify virus strains and perhaps epidemiology, or determine evolution of various different viruses.

This invention also provides reagents with significant therapeutic value. The UL144 (naturally occurring or recombinant), fragments thereof, and antibodies thereto, along with compounds identified as having binding affinity to UL144, should be useful in the treatment of conditions associated with abnormal physiology or development, including abnormal proliferation, e.g., cancerous conditions, or degenerative conditions. In particular, modulation of development of lymphoid cells will be achieved by appropriate therapeutic treatment using the compositions provided herein. For example, a disease or disorder associated with abnormal expression of UL144 should be a likely target for an antagonist which prevents the blockage of the death domain receptor signaling. The antigen plays a role in regulation or development of target cells, e.g., virus targets.

In particular, the antigen may provide a costimulatory signal to cell activation, or be involved in regulation of cell proliferation or differentiation. Thus, the UL144 will likely modulate cells which possess a receptor therefor, e.g., T cell mediated interactions with other cell types. These interactions would lead, in particular contexts, to modulation of apoptosis, cell growth, cytokine synthesis by those or other cells, or development of particular effector cells.

Moreover, the UL144 or antagonists could affect apoptosis, which may be induced by virus infection. Among these agonists should be various antibodies which recognize the appropriate epitopes, e.g., which mimic binding of ligand to its death domain receptor.

Alternatively, they may bind to epitopes which sterically can block receptor binding.

UL144 may be important in regulation of virus infection and replication. See, e.g., Richman, et al. (eds. 1997) Clinical Virology Churchill-Livingstone; Fields, et al. (eds. 1995) Fundamental Virology Raven Press, NY. Experiments show that the presence of UL144 affects apoptosis pathways, and that UL144 can be introduced into cells to sustain and prevent certain apoptotic signals. Alternatively, various antibodies should serve to block the UL144 effect.

UL144 antibodies can be purified and then administered to a patient, veterinary or human. These reagents can be combined for therapeutic use with additional active or inert ingredients, e.g., in conventional pharmaceutically acceptable carriers or diluents, e.g., immunogenic adjuvants, along with physiologically innocuous stabilizers, excipients, or preservatives. These combinations can be sterile filtered and placed into dosage forms as by lyophilization in dosage vials or storage in stabilized aqueous preparations. This invention also contemplates use of antibodies or binding fragments thereof, including forms which are not complement binding.

Drug screening using UL144 or fragments thereof can be performed to identify compounds having binding affinity to or other relevant biological effects on receptor functions, including isolation of associated components. Subsequent biological assays can then be utilized to determine if the compound has intrinsic stimulating activity or is a blocker or antagonist in that it blocks the activity of the antigen, e.g., mutein antagonists. Likewise, a compound having intrinsic stimulating activity can activate the signal pathway, e.g., cytotoxic death signal, antiviral activity, or induction of nitric oxide synthase activity, and is thus an agonist in that it can overcome any blocking activity of these soluble forms of receptors. This invention

further contemplates the therapeutic use of blocking antibodies to UL144 as agonists or antagonists and of stimulatory molecules, e.g., muteins, as agonists. This approach should be particularly useful with other soluble receptor species variants. In particular, screening for compounds which block the formation of inactive death domain receptors incorporating the UL144 is made available.

The quantities of reagents necessary for effective therapy will depend upon many different factors, including means of administration, target site, physiological state of the patient, and other medicants administered. Thus, treatment dosages should be titrated to optimize safety and efficacy. Typically, dosages used in vitro may provide useful guidance in the amounts useful for in situ administration of these reagents. Animal testing of effective doses for treatment of particular disorders will provide further predictive indication of human dosage. Various considerations are described, e.g., in Gilman, et al. (eds. 1998) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 9th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn. Methods for administration are discussed therein and below, e.g., for oral, intravenous, intraperitoneal, or intramuscular administration, transdermal diffusion, and others. Pharmaceutically acceptable carriers will include water, saline, buffers, and other compounds described, e.g., in the Merck Index, Merck & Co., Rahway, New Jersey. Dosage ranges would ordinarily be expected to be in amounts lower than 1 mM concentrations, typically less than about 10 μM concentrations, usually less than about 100 nM, preferably less than about 10 pM (picomolar), and most preferably less than about 1 fM (femtomolar), with an appropriate carrier. Slow release formulations, or a slow release apparatus will often be utilized for

continuous or long term administration. See, e.g., Langer (1990) Science 249:1527-1533.

UL144, fragments thereof, and antibodies to it or its fragments, antagonists, and agonists, may be administered directly to the host to be treated or, depending on the size of the compounds, it may be desirable to conjugate them to carrier proteins such as ovalbumin or serum albumin prior to their administration. Therapeutic formulations may be administered in many conventional dosage formulations. While it is possible for the active ingredient to be administered alone, it is preferable to present it as a pharmaceutical formulation. Formulations typically comprise at least one active ingredient, as defined above, together with one or more acceptable carriers thereof. Each carrier should be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the patient. Formulations include those suitable for oral, rectal, nasal, topical, or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. See, e.g., Gilman, et al. (eds. 1998) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 9th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn.; Avis, et al. (eds. 1993) Pharmaceutical Dosage Forms: Parenteral Medications, Dekker, New York; Lieberman, et al. (eds. 1990) Pharmaceutical Dosage Forms: Tablets, Dekker, New York; and Lieberman, et al. (eds. 1990) Pharmaceutical Dosage Forms: Disperse Systems, Dekker, New York. The therapy of this invention may be combined with or used in association with other agents, e.g., other antiviral or antimicrobial agents or therapeutic entities, e.g., antibiotics.

Both the naturally occurring and the recombinant forms of the UL144 of this invention are particularly useful in kits and assay methods which are capable of screening compounds for binding activity to the proteins. Several methods of automating assays have been developed in recent years so as to permit screening of tens of thousands of compounds in a short period. See, e.g., Fodor, et al. (1991) Science 251:767-773, which describes means for testing of binding affinity by a plurality of defined polymers synthesized on a solid substrate. The development of suitable assays can be greatly facilitated by the availability of large amounts of purified, soluble UL144 as provided by this invention.

Other methods can be used to determine the critical residues in the UL144-ligand interactions. Mutational analysis can be performed, e.g., see Somoza, et al. (1993) J. Exp. Med. 178:549-558, to determine specific residues critical in the interaction and/or signaling with other components of a receptor complex, both extracellular domains, involved in the soluble ligand interaction, or intracellular domain of a transmembrane form, which provides interactions important in intracellular signaling.

For example, antagonists can normally be found once the antigen has been structurally defined, e.g., by tertiary structure data. Testing of potential interacting analogs is now possible upon the development of highly automated assay methods using a purified UL144. In particular, new agonists and antagonists will be discovered by using screening techniques described herein. Of particular importance are compounds found to have a combined binding affinity for a spectrum of UL144 molecules, e.g., compounds which can serve as antagonists for strain or species variants of UL144.

One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant DNA molecules expressing a UL144. Cells may

be isolated which express a UL144 in isolation from other molecules. Such cells, either in viable or fixed form, can be used for standard binding partner binding assays. See also, Parce, et al. (1989) Science 246:243-247; and Owicki, et al. (1990) Proc. Nat'l Acad. Sci. USA 87:4007-4011, which describe sensitive methods to detect cellular responses.

Another technique for drug screening involves an approach which provides high throughput screening for compounds having suitable binding affinity to a UL144 and is described in detail in Geysen, European Patent Application 84/03564, published on September 13, 1984. First, large numbers of different small peptide test compounds are synthesized on a solid substrate, e.g., plastic pins or some other appropriate surface, see Fodor, et al. (1991). Then all the pins are reacted with solubilized, unpurified or solubilized, purified UL144, and washed. The next step involves detecting bound UL144.

Rational drug design may also be based upon structural studies of the molecular shapes of the UL144 and other effectors or analogs. Effectors may be other proteins which mediate other functions in response to binding, or other proteins which normally interact with UL144. One means for determining which sites interact with specific other proteins is a physical structure determination, e.g., x-ray crystallography or 2 dimensional NMR techniques. These will provide guidance as to which amino acid residues form molecular contact regions. For a detailed description of protein structural determination, see, e.g., Blundell and Johnson (1976) Protein Crystallography, Academic Press, New York.

IX. Kits

This invention also contemplates use of UL144 proteins, fragments thereof, peptides, and their fusion products in a variety of diagnostic kits and methods for

detecting, e.g., the presence of a UL144 or binding partner. Typically the kit will have a compartment containing either a defined UL144 peptide or gene segment or a reagent which recognizes one or the other, e.g., UL144 fragments or antibodies.

A kit for determining the binding affinity of a test compound to, e.g., a UL144, would typically comprise a test compound; a labeled compound, for example a binding partner or antibody having known binding affinity for UL144; a source of UL144 (naturally occurring or recombinant); and a means for separating bound from free labeled compound, such as a solid phase for immobilizing the molecule. Once compounds are screened, those having suitable binding affinity to the antigen can be evaluated in suitable biological assays, as are well known in the art, to determine whether they act as agonists or antagonists to the death domain signaling pathway, including, e.g., cytotoxic death signal transmission, antiviral effects, e.g., of infection or replication, or induction of nitric oxide synthase activity. The availability of recombinant UL144 polypeptides also provide well defined standards for calibrating such assays.

A preferred kit for determining the concentration of, e.g., a UL144 in a sample would typically comprise a labeled compound, e.g., binding partner or antibody, having known binding affinity for the antigen, a source of antigen (naturally occurring or recombinant) and a means for separating the bound from free labeled compound, e.g., a solid phase for immobilizing the UL144. Compartments containing reagents, and instructions, will normally be provided.

Antibodies, including antigen binding fragments, specific for, e.g., the UL144 or fragments, are useful in diagnostic applications to detect the presence of elevated levels of UL144 and/or its fragments. Such diagnostic assays can employ lysates, live cells, fixed cells, immunofluorescence, cell cultures, body fluids,

and further can involve the detection of antigens related to the antigen in serum, or the like. Diagnostic assays may be homogeneous (without a separation step between free reagent and antigen-binding partner complex) or heterogeneous (with a separation step). Various commercial assays exist, such as radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), enzyme-multiplied immunoassay technique (EMIT), substrate-labeled fluorescent immunoassay (SLFIA), and the like. See, e.g., Van Vunakis, et al. (1980) Meth Enzymol. 70:1-525; Harlow and Lane (1980) Antibodies: A Laboratory Manual, CSH Press, NY; and Coligan, et al. (eds. 1993) Current Protocols in Immunology, Greene and Wiley, NY.

Anti-idiotypic antibodies may have similar use to diagnose presence of antibodies against a UL144, as such may be diagnostic of various abnormal states. For example, overproduction of UL144 may result in production of various immunological reactions which may be diagnostic of abnormal physiological states, particularly in proliferative cell conditions such as cancer or abnormal activation or differentiation.

Frequently, the reagents for diagnostic assays are supplied in kits, so as to optimize the sensitivity of the assay. For the subject invention, depending upon the nature of the assay, the protocol, and the label, either labeled or unlabeled antibody or binding partner, or labeled UL144 is provided. This is usually in conjunction with other additives, such as buffers, stabilizers, materials necessary for signal production such as substrates for enzymes, and the like. Preferably, the kit will also contain instructions for proper use and disposal of the contents after use. Typically the kit has compartments for each useful reagent. Desirably, the reagents are provided as a dry lyophilized powder, where the reagents may be

reconstituted in an aqueous medium providing appropriate concentrations of reagents for performing the assay.

Many of the aforementioned constituents of the drug screening and the diagnostic assays may be used without modification or may be modified in a variety of ways. For example, labeling may be achieved by covalently or non-covalently joining a moiety which directly or indirectly provides a detectable signal. In many of these assays, the binding partner, test compound, UL144, or antibodies thereto can be labeled either directly or indirectly. Possibilities for direct labeling include label groups: radiolabels such as ^{125}I, enzymes (U.S. Pat. No. 3,645,090) such as peroxidase and alkaline phosphatase, and fluorescent labels (U.S. Pat. No. 3,940,475) capable of monitoring the change in fluorescence intensity, wavelength shift, or fluorescence polarization. Possibilities for indirect labeling include biotinylation of one constituent followed by binding to avidin coupled to one of the above label groups.

There are also numerous methods of separating the bound from the free UL144, or alternatively the bound from the free test compound. The UL144 can be immobilized on various matrixes followed by washing. Suitable matrixes include plastic such as an ELISA plate, filters, and beads. See, e.g., Coligan, et al. (eds. 1993) Current Protocols in Immunology, Vol. 1, Chapter 2, Greene and Wiley, NY. Other suitable separation techniques include, without limitation, the fluorescein antibody magnetizable particle method described in Rattle, et al. (1984) Clin. Chem. 30:1457-1461, and the double antibody magnetic particle separation as described in U.S. Pat. No. 4,659,678.

Methods for linking proteins or their fragments to the various labels have been extensively reported in the literature and do not require detailed discussion here. Many of the techniques involve the use of activated carboxyl groups either through the use of carbodiimide

or active esters to form peptide bonds, the formation of thioethers by reaction of a mercapto group with an activated halogen such as chloroacetyl, or an activated olefin such as maleimide, for linkage, or the like. Fusion proteins will also find use in these applications.

Another diagnostic aspect of this invention involves use of oligonucleotide or polynucleotide sequences taken from the sequence of UL144. These sequences can be used as probes for detecting levels of the UL144 message in samples from patients suspected of having infection of a CMV strain, and perhaps to determine the virulence of the infective strain. Since the antigen is a marker for highly virulent strains, it may be useful to evaluate the infective strain and follow its epidemiology. The preparation of both RNA and DNA nucleotide sequences, the labeling of the sequences, and the preferred size of the sequences has received ample description and discussion in the literature. See, e.g., Langer-Safer, et al. (1982) Proc. Nat'l. Acad. Sci. 79:4381-4385; Caskey (1987) Science 236:962-967; and Wilchek et al. (1988) Anal. Biochem. 171:1-32.

Diagnostic kits which also test for the qualitative or quantitative presence of other markers are also contemplated. Diagnosis or prognosis may depend on the combination of multiple indications used as markers. Thus, kits may test for combinations of markers. See, e.g., Viallet, et al. (1989) Progress in Growth Factor Res. 1:89-97.

X. Methods for Isolating TNF-R Specific Binding Partners

The UL144 protein should interact with other death domain receptor subunits based, e.g., upon its similarity in structure and function to other cell surface antigens exhibiting similar structure and cell type specificity of expression. Methods to isolate an

interacting subunit are made available by the ability to make purified UL144 for screening programs. Sequences provided herein will allow for screening or isolation of specific receptor subunits. Many methods exist for expression cloning, panning, affinity isolation, or other means to identify an interacting subunit. A two-hybrid selection system may also be applied making appropriate constructs with the available UL144 sequences. See, e.g., Fields and Song (1989) Nature 340:245-246.

The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the invention to specific embodiments.

EXAMPLES

General Methods

Some of the standard methods are described or referenced, e.g., in Maniatis, et al. (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor Press; Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual (2d ed.), vols. 1-3, CSH Press, NY; Ausubel, et al., Biology, Greene Publishing Associates, Brooklyn, NY; or Ausubel, et al. (1987 and Supplements) Current Protocols in Molecular Biology, Greene and Wiley, New York; Innis, et al. (eds.)(1990) PCR Protocols: A Guide to Methods and Applications, Academic Press, N.Y. Methods for protein purification include such methods as ammonium sulfate precipitation, column chromatography, electrophoresis, centrifugation, crystallization, and others. See, e.g., Ausubel, et al. (1987 and periodic supplements); Deutscher (1990) "Guide to Protein Purification" in Methods in Enzymol. vol. 182, and other volumes in this series; and manufacturer's literature on use of protein purification products, e.g., Pharmacia, Piscataway, N.J., or Bio-Rad, Richmond, CA. Combination with recombinant techniques allow fusion to appropriate

segments, e.g., to a FLAG sequence or an equivalent which can be fused via a protease-removable sequence. See, e.g., Hochuli (1989) Chemische Industrie 12:69-70; Hochuli (1990) "Purification of Recombinant Proteins with Metal Chelate Absorbent" in Setlow (ed.) Genetic Engineering, Principle and Methods 12:87-98, Plenum Press, N.Y.; and Crowe, et al. (1992) QIAexpress: The High Level Expression & Protein Purification System QIAGEN, Inc., Chatsworth, CA. Cell culture techniques are described in Doyle, et al. (eds. 1994) Cell and Tissue Culture: Laboratory Procedures, John Wiley and Sons, NY.

Standard immunological techniques are described, e.g., in Hertzenberg, et al. (eds. 1996) Weir's Handbook of Experimental Immunology vols. 1-4, Blackwell Science; Coligan (1991) Current Protocols in Immunology Wiley/Greene, NY; and Methods in Enzymology volumes. 70, 73, 74, 84, 92, 93, 108, 116, 121, 132, 150, 162, and 163.

FACS analyses are described in Melamed, et al. (1990) Flow Cytometry and Sorting Wiley-Liss, Inc., New York, NY; Shapiro (1988) Practical Flow Cytometry Liss, New York, NY; and Robinson, et al. (1993) Handbook of Flow Cytometry Methods Wiley-Liss, New York, NY. Fluorescent labeling of appropriate reagents was performed by standard methods.

Cell lines and reagents

The human leukemia T cell line Jurkat, the carcinoma line HeLa and the human fibroblasts were all maintained in RPMI (Gibco) supplemented with 10% FBS (Gibco). Stable transfectants of HeLa and Jurkat cells were selected and maintained in 2.5 mg/ml of G418 (Gibco). Recombinant FAS-L, TRAIL, and TNF-α were purchased commercially (Alexis, San Diego). DX21 is a mouse IgG1 mAb directed at FAS, which induces apoptosis without the need for second step cross-linking.

Constructs and Transfection

An epitope tagged UL144 beginning at aa 21 of the mature protein was constructed using the FLAG tag (DYKDDDDK) downstream of the CD8-signal peptide. The FLAG-UL144 was subcloned into pEF-BOS or pMXneo. Stable Jurkat cell transfectants were generated by sorting FLAG positive cells, which were generated either by retroviral infection (pMXneo UL144) or electroporation (pEF-BOS UL144). The truncation mutant of UL144 was generated by incorporating a stop codon at amino acid 161 (aga->taa) by PCR and the sequence was confirmed by sequencing. Jurkat cells were infected with retrovirus generated using the Φ-NX-A amphotropic packing line (a gift from G. Nolan, Stanford University). 293 cells were plated at 2 x 10^5 cells/well and transfected with Lipofectamine according to the manufacturers instructions. Cells were harvested 30-40 h later and assessed for apoptosis.

293 cells were plated at 8 x 10^6 cells per T75 flask and transfected 8 h later by Lipofectamine (Gibco) for 16 h in Opti-MEM media (Gibco), followed by regular media for a further 24 h and then harvested for co-immunoprecipitations.

Immunoprecipitation.

1 x 10^7 cells were washed twice in PBS and lysed in 0.5 ml of Lysis Buffer (TBST with 1% NP40, 1 mM PMSF, and protease inhibitors). Lysates were immunoprecipitated with Protein G coupled M2 for 2 h at room temperature, washed five-times with lysis buffer, and eluted. Boiled samples were analyzed by Western Blot, probed with Biotinylated M2 (anti-FLAG) or biotinylated-DX2 (anti-FAS) followed by streptavidin-HFP (Amersham) and developed with Pierce SuperSignal ULTRA Chemiluminescent substrate (Pierce, IL).

Apoptosis Assays

Stably transfected Jurkat cells were plated at 2 x 10^4 cells/well (Jurkat) with recombinant FAS-L (Alexis,

San Diego) or TRAIL (Alexis, San Diego) over a range of concentrations. Cells were cultured for 16 h at 37° C. Stably transfected HeLa cells were plated at 3 x 10^4 cells/well with 10 µg/ml cycloheximide for 6 h, followed by the addition of recombinant TNF-α (Promega). Cell viability was quantified using Celltiter 96AQ Proliferation Assay (Promega). Apoptotic cells were quantitated by staining with Annexin V and Propidium Iodide (Pharmingen, San Diego).

HCMV infection

The AD169 and Toledo strains of HCMV were titered and propagated in HFF. Mocarski, et al. (1993) Proc. Nat'l Acad. Sci. USA 90:104-108. HFF were infected at MOI 3-5. After adsorption of the virus for 1 h at 37° C the inoculum was removed and replaced with medium containing 10% FCS. Viral stocks were titered using classical cytopathic effect as an end-point. Total RNA were isolated from 10^7 infected HF at each time point using RNAzol B (Tell-Test, Tx). Northern Blots were probed using standard methodology.

EXAMPLE 1: Ligand Induced Apoptosis

Normal human fibroblasts (HF) are not susceptible to cell-death triggered by either FAS-L or TRAIL; however, upon infection with the AD169 strain of HCMV, HF are rendered susceptible. Interestingly, HF infected with a more recent clinical isolate of HCMV, the Toledo strain, are resistant to both FAS-L or TRAIL induced apoptosis. Recent clinical isolates of HCMV are known to contain genes that are not present in AD169 and Towne; the Toledo isolate contains 19 additional open-reading frames (ORF) absent in AD169. Cha, et al. (1996) J. Virol. 70:78-83. The differences in susceptibility between Toledo and AD169 to FAS-L and TRAIL may be due to one or several of these ORF. ORF UL144 is one of the 19 Toledo genes absent in AD169; this ORF encodes a protein with homology to members of

the TNF-Receptor family. Since FAS and TRAIL both belong to the TNF-R family, UL144 was a good candidate for a gene that could modulate susceptibility to apoptosis.

HF cells infected with AD169, but not Toledo, become susceptible to FAS-L and TRAIL induced apoptosis. HF were infected at MOI 5 with AD169 or Toledo, or mock infected. Cells were assayed from 24-96 h post-infection. Cells infected with AD169 acquire susceptibility to FAS-L and TRAIL after 48 h post-infection. 2 x 10^5 HF were cultured with 2 µg/ml of TRAIL or 5 µg/ml of anti-FAS (DX21) in 0.5 ml of media, left on ice for 10 minutes, and cultured overnight at 37° C. Apoptosis was assessed by staining with Annexin V and PI.

EXAMPLE 2: Transcript analysis

Northern analysis of total RNA from HF infected with Toledo show that UL144 produces a 1 kb transcript that can be detected 24 h post infection and accumulates as the infection progresses. No such transcript was detected in RNA from AD169.

Northern analysis was performed using ten micrograms of total RNA isolated from Toledo infected HF and probed with ^{32}P labeled random-primed cDNA probes of UL144.

EXAMPLE 3: UL144 Protection From Induced Apoptosis

The human leukemia cell line Jurkat is susceptible to FAS-L and TRAIL induced apoptosis; while the human carcinoma cell HeLa is susceptible to TNF-α triggered apoptosis (in the presence of cycloheximide). To test the protective effects of UL144, stable transfectants were generated in Jurkat T cells and HeLa cells.

Jurkat were stably transduced the pMXneo retroviral construct containing a FLAG-tagged UL144. HeLa cells were transfected using Lipofectamine with FLAG-tagged

UL144 in pEF-BOS vector. Positive pools of cells were enriched by several rounds of FACS sorting. Transfectants were stained with cIg or M2 followed by PE conjugated goat anti-mouse Ig. These results showed that FLAG-tagged UL144 can be detected on the cell surface of both transfectants.

UL144 protects transfectants against FAS-L and TRAIL induced apoptosis. Parental Jurkat cells, UL144 transfected Jurkat cells, and vector controlled transfected Jurkat cells were incubated with the indicated amounts of recombinant FAS-L for 20 h at 37° C. Cell viability was subsequently determined using a cell proliferation assay. Where indicated cells were preincubated with zVAD (100 μM) for 3 h at 37° C. Staurosporine was used at 1 μg/ml. These results showed that Jurkat cells transfected with UL144 were less susceptible to FAS-L induced apoptosis compared to parental Jurkat or vector only control transfectants. All cell lines are equally susceptible to apoptosis induced via Staurosporine, which uses an independent apoptotic pathway.

UL144 protects transfectants against TNF-α induced apoptosis. Parental HeLa cells, UL144 transfected HeLa cells and vector controlled transfected HeLa cells were incubated with the indicated amounts of recombinant TNF-α for 20 h at 37° C. Cell viability was subsequently determined using a cell proliferation assay. Where indicated cells were preincubated with zVAD (100 μM) for 3 h at 37° C. Staurosporine was used at 1 μg/ml. These results showed that treatment of Jurkat cells with the general caspase inhibitor zVAD-fmk prevents cell death. UL144 Jurkat transfectants are also more resistant to TRAIL-induced cell death. The expression of UL144 protects HeLa cells from TNF-α induced apoptosis.

Therefore, UL144 expression can protect cells against apoptosis triggered by at least three different death-domain containing receptors. The anti-apoptotic activity of UL144 correlated with the relative level of

UL144 compared to the death-receptor (i.e., FAS or TNF-R1). In Jurkat cells, which express high levels of FAS, transfectants that expressed UL144 levels comparable to FAS levels were only marginally protective, significant protection was only seen when transfectants were sorted for levels of UL144 in excess of endogenous FAS. In HeLa cells, which express only low levels of TNF-R1, expression of UL144 conferred almost complete protection.

EXAMPLE 4: Mechanistic studies

There are at least two obvious mechanisms that can account for the inhibitory effects of UL144. In the first, the extracellular region of UL144 may prevent efficient ligand binding to FAS, TRAIL-R, or TNF-R1. This is unlikely, however, because UL144 prevents apoptosis induced by anti-FAS mAb (as opposed to FAS-L binding), even though mAb binding is un-affected. In the second, UL144 may function as a dominant negative-signaling receptor. UL144 may become incorporated into the receptor complex, but because it lacks a proper death-domain, it is incapable of recruiting the substrates required to trigger apoptosis.

To address whether UL144 is capable of forming complexes with FAS, 293 cells were transiently transfected with FAS alone, or FAS together with UL144. 293 cells were transfected with expression vectors with FAS or FAS with FLAG-UL144. Cell extracts were immunoprecipitated with control Ig or anti-FLAG mAB (M2). Western Blots were performed with Biotinylated M2.

Immunoprecipitation of FAS also precipitates UL144, or vice versa. This suggests that the cytoplasmic domain of UL144 plays a critical role in allowing its incorporation into death-receptor complexes. If this was the case, a truncation mutant of UL144 without the cytoplasmic domain should not be able to prevent

apoptosis. A UL144 cytoplasmic-tail truncation mutant was generated and stably transfected into Jurkat.

Jurkat transfected with full-length or truncated UL144 were cultured with FAS-L (50 ng/ml) or TRAIL (50 ng/ml) for 20 h at 37° C. The percentage of apoptotic cells were determined by FACS by gating on the number of Annexin/PI positive cells. The results showed that transfectants expressing wild-type UL144 protected the transfectants against both FAS-L and TRAIL induced apoptosis, however, no protection was seen with transfectants expressing the truncated UL144 gene.

Point mutations in the death-domain of FAS or mutations arising in human Autoimmune Lymphoproliferative Syndrome (ALPS) patients result in the inability of FAS to recruit the adapter molecule FADD. Huang, et al. (1996) Nature 384:638-641. FAS association to FADD is required for the recruitment of proximal caspases, e.g., caspase 8. A defective FAS possibly prevents the recruitment of sufficient adapter molecules (FADD) necessary to initiate the activation of proximal caspases (e.g., caspases 8 and/or 10), which are required to initiate the apoptotic cascade. If UL144 functions in a similar fashion by blocking adapter molecule recruitment, then apoptosis should still be induced by a chimeric FAS-FLICE (caspase 8) fusion protein, which bypasses the need to recruit intermediate adapter molecules. 293 cells were transfected with combinations of UL144 with FAS or the FAS-FLICE chimeric construct.

Protection from FAS-FLICE chimeric protein

293 cells were transfected with FAS with UL144, or FAS-FLICE with UL144, both in the presence of Green Fluorescent Protein (GFP). 30 h post-transfection cells were stained with annexin-PE and PI and gated for GFP positive cells. These results showed that UL144 only protects against FAS, but not the FAS-FLICE chimera,

demonstrating that UL144 functions by preventing FLICE recruitment by the death-receptors.

Viruses have developed an impressive array of molecules that can undermine critical points in apoptosis signaling. The Adenovirus RID protein results in the degradation of cellular FAS (Tollefson, et al. (1998) Nature 392:726-730); while the Myxoma virus expresses a soluble TNF-R homolog (T2) that competes with cell surface TNF-R for ligand binding (Schrieber, et al. (1997) J. Virol. 71:2171-2181). Ligand binding to TNF-R superfamily members results in their multimerization and subsequent recruitment of adapter and catalytic molecules. Multimerization of FAS recruits FADD via interactions of the death domains (DD) in the two molecules. Chinnaiyan, et al. (1995) Cell 81:505-512. FADD expresses a second domain termed the death effector domain (DED), which allows proximal caspases (e.g., caspase 8 and 10) to be incorporated into the FAS death complex. Kischel, et al. (1995) EMBO J. 14:5579-5588. The Molluscum contagiosum virus (MCV) protein MC159 contains two DED that bind to FADD preventing the recruitment and activation of Caspase 8 (Thome, et al. (1997) Nature 386:517-521; and Bertin, et al. (1997) Proc. Nat'l Acad. Sci. USA 94:1172); while the equine herpesvirus type 2 E8 protein binds Caspase 8 (Hu, et al. (1997) J. Biol. Chem. 272:9621-9624) and prevents its incorporation into the death-inducing signaling complex (DISC) complex. Expression of both of these viral proteins abrogate the apoptotic effects of TNF-α, FAS-L, and TRAIL. Thome, et al. (1997) Nature 386:517-521; Bertin, et al. (1997) Proc. Nat'l Acad. Sci. USA 94:1172; and Hu, et al. (1997) J. Biol. Chem. 272:9621. The recruitment of Caspase 8 into the death complex initiates the activation of downstream caspases that are responsible for apoptosis. Cowpox virus and Baculovirus express proteins (crmA, p35, and iAP; Tewari and Dixit (1995) J. Biol. Chem. 270:3255-3260; and Clem

and Miller (1994) pages 89-110 in Tomei, et al. The Molecular Basis of Apoptosis in Disease Cold Spring Harbor Laboratory Press., New York) that interfere with Caspase 8 and downstream caspases (e.g., caspases 3, 6, and 7) are able to attenuate apoptotic signals via the death receptors (TNF-R1, FAS, DR3, TRAIL 1 and 2). The presence of TNF-R homologs have been described in several viruses, such as the Shope fibroma virus SFV-T2, Myxoma virus MYX-T2, Vaccinia Virus Va53 or SaIF19R, Variola virus G4R, and Cowpox virus crmB. Smith, et al. (1994) Cell 76:959-962. UL144 differs from these other homologs in that it is a transmembrane protein; and is capable of attenuating apoptosis induced by multiple ligands. There is a large body of evidence that the cytoplasmic domains of FAS and TNF-R1 spontaneously aggregate; we predict that the conserved motif found in UL144 and TNF-R1, FAS, and TRAMP is responsible for this aggregation. Boldin, et al. (1995) J. Biol. Chem. 270:387-391. In the absence of ligand, the receptor death-domains are prevented from spontaneously aggregating and triggering cell-death because the extracellular-domain of the receptors adopt a head-to-tail conformation which keeps the cytoplasmic regions apart. Naismith, et al. (1995) J. Biol. Chem. 270:13303-13307. In the presence of trimeric ligand, the whole complex becomes re-aligned allowing the death-domains to be brought together. It is suggested that the SxGKxxY motif found conserved in UL144 and the TRAIL receptors plays a role in allowing association of their cytoplasmic-domains. Schneider, et al. (1997) Immunity 7:831-836. Since TRAIL, TNF-α, and FAS-L are sufficiently different structurally that they do not functionally cross-react, the extracellular domains of UL144 are unlikely to play a role in binding these ligands. The function of UL144's extracellular domain may be to allow this viral molecule to interact with other members of the TNFR family.

The destruction of the infected cells prior to the completion of the viral replication cycle will significantly jeopardize the virus's ability to efficiently disseminate. AD169 infected HF become susceptible to apoptosis before the viral replication is completed (48 h) before the virus has completed its replication cycle (96 h). The absence of UL144 in AD169 and Towne strains of HCMV is therefore likely to contribute to the attenuated virulence of these strains.

EXAMPLE 5: Preparation of antibodies

Synthetic peptides or purified protein, natural or recombinant, are presented to an immune system to generate monoclonal or polyclonal antibodies. See, e.g., Coligan (1991) Current Protocols in Immunology Wiley/Greene; and Harlow and Lane (1989) Antibodies: A Laboratory Manual Cold Spring Harbor Press. Alternatively, adenovirus or other DNA expression methods may be used to express the desired polypeptides in situ, leading to immunization. Polyclonal serum, or hybridomas may be prepared. In appropriate situations, the binding reagent is either labeled as described above, e.g., fluorescence or otherwise, or immobilized to a substrate for panning methods.

All citations herein are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

WHAT IS CLAIMED IS:

1. A sustantially pure or recombinant polypeptide which:

a) binds to a polyclonal antibody which binds to the mature SEQ ID NO: 2; and

b) comprises at least two distinct segments of at least 8 contiguous amino acids of the mature SEQ ID NO: 2.

2. The polypeptide of Claim 1, wherein:

A) said segments include:

a) a third distinct segment of at least 8 contiguous amino acids;

b) at least one said segment of at least 12 amino acids; or

c) two of said segments of at least 10 amino acids each; or

B) said polypeptide:

a) in an in vitro Death Domain receptor assay, can block the response induced by its respective signaling ligand; or

b) can modulate virus induced apoptosis.

3. The polypeptide of Claim 2, wherein said death domain receptor is selected from:

a) Fas;

b) TNF-R1;

c) TRAMP;

d) DR6;

e) TRAIL-R1;

f) TRAIL-R2; or

g) TRAIL-R4.

4. The polypeptide of Claim 2B, wherein said assay is selected from:

a) a cytotoxic death signal;

b) an antiviral response; or

c) induction of nitric oxide synthase activity.

5. The polypeptide of Claim 1, which comprises a sequence of:

a) mature SEQ ID NO: 2;
b) residues 134-176 of SEQ ID NO: 2;
c) LAWLSLFIFLVGIILLILYLIAA (residues 134-156 of SEQ ID NO: 2);
d) YRSERC (residues 157-162 of SEQ ID NO: 2);
e) RCQQCC (residues 161-166 of SEQ ID NO: 2);
f) SIGKIFY (residues 167-173 of SEQ ID NO: 2); or
g) KIFYRTL (residues 170-176 of SEQ ID NO: 2).

6. A substantially pure or recombinant nucleic acid encoding the polypeptide of Claim 1.

7. The nucleic acid of Claim 6, comprising:

a) the mature polypeptide coding region of SEQ ID NO: 1;
b) at least 17 contiguous nucleotides of the mature polypeptide coding portion of SEQ ID NO: 1; or
c) at least 27 contiguous nucleotides of SEQ ID NO: 1.

8. An expression vector comprising sequence which hybridizes at 55 degrees centigrade and 400 mM salt to the nucleic acid of Claim 7a.

9. The vector of Claim 8, which hybridizes at 65 degrees centigrade and/or 200 mM salt.

10. The nucleic acid of Claim 6, wherein said 17 contiguous nucleotides are from:

a) nucleotides 400-468 of SEQ ID NO: 1;
b) nucleotides 469-528 of SEQ ID NO: 1; or
c) nucleotides 400-528 of SEQ ID NO: 1.

11. A method of modulating apoptosis of a cell, comprising exposing said cell to a sufficient amount of:

a) a polypeptide of Claim 1, thereby protecting said cell; or

b) a blocking compound which blocks the effect of UL144 on apoptosis.

12. The method of Claim 11, wherein said apoptosis is mediated by a TNF-R family member.

13. The method of Claim 12, wherein said apoptosis is induced by a TNF family member selected from TNF-α, FAS ligand, TRAIL, or TRAMP.

14. The method of Claim 11, wherein:

a) said modulating is reducing, and said polypeptide is administered in combination with:

i) an immunostimulant;

ii) an antitumor reagent; or

iii) an antitumor treatment, including radiation therapy; or

b) said modulating is increasing, said blocking compound is a monoclonal antibody or antisense polynucleotide, and said blocking compound is administered in combination with:

i) an antimicrobial agent;

ii) an immune adjuvant; or

iii) an antitumor treatment.

15. A substantially pure binding compound comprising an antigen binding site from an antibody, wherein said antibody binds selectively to a polypeptide of Claim 1.

16. The binding compound of Claim 15, wherein:

a) said antibody is raised against a polypeptide of Claim A5; or

b) said antibody is raised against a polypeptide comprising the mature SEQ ID NO: 2.

17. The binding compound of Claim 15, which:

a) is immunoselected;
b) is a polyclonal antibody;
c) binds to a denatured protein from SEQ ID NO: 2;
d) exhibits a Kd to antigen of at least 30 mM;
e) is attached to a solid substrate, including a bead or plastic membrane;
f) is in a sterile composition; or
g) is detectably labeled, including a radioactive or fluorescent label.

18. A method of screening for a molecule which blocks a TNF family member induced apoptosis, said method comprising screening a compound library comprising a polypeptide of Claim 1 for the ability to block said apoptosis.

19. A method of screening for a molecule which interferes with UL144 anti-apoptotic activity, said method comprising screening a compound for its ability to block UL144 antiapoptotic activity.

20. The method of Claim 19, wherein said compound is selected from:

a) an antibody which binds UL144; or
b) an antisense nucleic acid comprising sequence complementary to a nucleic acid which encodes UL144.

SEQUENCE LISTING

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1               5                  10                  15

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Leu Gly Val Thr Lys Val Cys Gln His Asn Glu Val Gln Leu Gly Asn
            20                  25                  30

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Glu Cys Cys Pro Pro Cys Gly Ser Gly Gln Arg Val Thr Lys Val Cys
        35                  40                  45

ACG GAT TAT ACC AGT GTA ACG TGT ACC CCT TGC CCC AAC GGC ACG TAT     192
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    50                  55                  60

GTA TCG GGA CTT TAC AAC TGT ACC GAT TGC ACT CAA TGT AAC GTC ACT     240
Val Ser Gly Leu Tyr Asn Cys Thr Asp Cys Thr Gln Cys Asn Val Thr
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Gln Val Met Ile Arg Asn Cys Thr Ser Thr Asn Asn Thr Val Cys Ala
                85                  90                  95

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Pro Lys Asn His Thr Tyr Phe Ser Thr Pro Gly Val Gln His His Lys
            100                 105                 110

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Gln Arg Gln Gln Asn His Thr Ala His Ile Thr Val Lys Gln Gly Lys
        115                 120                 125

AGC GGT CGT CAT ACT CTA GCC TGG TTG TCT CTC TTT ATC TTT CTT GTG     432
Ser Gly Arg His Thr Leu Ala Trp Leu Ser Leu Phe Ile Phe Leu Val
    130                 135                 140

GGT ATC ATA CTT TTA ATT CTC TAT CTT ATA GCC GCC TAT CGG AGT GAG     480
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145                 150                 155                 160
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            20                  25                  30

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        35                  40                  45

Thr Asp Tyr Thr Ser Val Thr Cys Thr Pro Cys Pro Asn Gly Thr Tyr
    50                  55                  60

Val Ser Gly Leu Tyr Asn Cys Thr Asp Cys Thr Gln Cys Asn Val Thr
65                  70                  75                  80

Gln Val Met Ile Arg Asn Cys Thr Ser Thr Asn Asn Thr Val Cys Ala
                85                  90                  95

Pro Lys Asn His Thr Tyr Phe Ser Thr Pro Gly Val Gln His His Lys
            100                 105                 110

Gln Arg Gln Gln Asn His Thr Ala His Ile Thr Val Lys Gln Gly Lys
        115                 120                 125

Ser Gly Arg His Thr Leu Ala Trp Leu Ser Leu Phe Ile Phe Leu Val
    130                 135                 140

Gly Ile Ile Leu Leu Ile Leu Tyr Leu Ile Ala Ala Tyr Arg Ser Glu
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            20                  25                  30

Lys Gly Leu Glu Leu Arg Lys Thr Val Thr Thr Val Glu Thr Gln Asn
        35                  40                  45

Leu Glu Gly Leu His His Asp Gly Gln Phe Cys His Lys Pro Cys Pro
    50                  55                  60

Pro Gly Glu Arg Lys Ala Arg Asp Cys Thr Val Asn Gly Asp Glu Pro
65                  70                  75                  80
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Asp Cys Val Pro Cys Gln Glu Gly Lys Glu Tyr Thr Asp Lys Ala His
                85                  90                  95

Phe Ser Ser Lys Cys Arg Arg Cys Arg Leu Cys Asp Glu Gly His Gly
            100                 105                 110

Leu Glu Val Glu Ile Asn Cys Thr Arg Thr Gln Asn Thr Lys Cys Arg
        115                 120                 125

Cys Lys Pro Asn Phe Phe Cys Asn Ser Thr Val Cys Glu His Cys Asp
    130                 135                 140

Pro Cys Thr Lys Cys Glu His Gly Ile Ile Lys Glu Cys Thr Leu Thr
145                 150                 155                 160

Ser Asn Thr Lys Cys Lys Glu Glu Gly Ser Arg Ser Asn Leu Gly Trp
                165                 170                 175

Leu Cys Leu Leu Leu Leu Pro Ile Pro Leu Ile Val Trp Val Lys Arg
            180                 185                 190

Lys Glu Val Gln Lys Thr Cys Arg Lys His Arg Lys Glu Asn Gln Gly
        195                 200                 205

Ser His Glu Ser Pro Thr Leu Asn Pro Glu Thr Val Ala Ile Asn Leu
    210                 215                 220

Ser Asp Val Asp Leu Ser Lys Tyr Ile Thr Thr Ile Ala Gly Val Met
225                 230                 235                 240

Thr Leu Ser Gln Val Lys Gly Phe Val Arg Lys Asn Gly Val Asn Glu
                245                 250                 255

Ala Lys Ile Asp Glu Ile Lys Asn Asp Asn Val Gln Asp Thr Ala Glu
            260                 265                 270

Gln Lys Val Gln Leu Leu Arg Asn Trp His Gln Leu His Gly Lys Lys
        275                 280                 285

Glu Ala Tyr Asp Thr Leu Ile Lys Asp Leu Lys Lys Ala Asn Leu Cys
    290                 295                 300

Thr Leu Ala Glu Lys Ile Gln Thr Ile Ile Leu Lys Asp Ile Thr Ser
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            20                  25                  30

His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
        35                  40                  45

Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
    50                  55                  60

Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
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Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
85 90 95

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100 105 110

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Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
130 135 140

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        35                  40                  45

Arg Gly Cys Pro Ala Gly His Tyr Leu Lys Ala Pro Cys Thr Glu Pro
    50                  55                  60

Cys Gly Asn Ser Thr Cys Leu Val Cys Pro Gln Asp Thr Phe Leu Ala
65                  70                  75                  80

Trp Glu Asn His His Asn Ser Glu Cys Ala Arg Cys Gln Ala Cys Asp
                85                  90                  95

Glu Gln Ala Ser Gln Val Ala Leu Glu Asn Cys Ser Ala Val Ala Asp
            100                 105                 110

Thr Arg Cys Gly Cys Lys Pro Gly Trp Phe Val Glu Cys Gln Val Ser
        115                 120                 125

Gln Cys Val Ser Ser Ser Pro Phe Tyr Cys Gln Pro Cys Leu Asp Cys
    130                 135                 140

Gly Ala Leu His Arg His Thr Arg Leu Leu Cys Ser Arg Arg Asp Thr
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Ala Val Gln Ser Ala Val Pro Leu Ser Val Ala Gly Gly Arg Val Gly
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Val Phe Trp Val Gln Val Leu Leu Ala Gly Leu Val Val Pro Leu Leu
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465                 470                 475                 480

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                485                 490                 495

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            500                 505                 510

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Cys Asn Arg Cys Thr Glu Gly Val Gly Tyr Thr Asn Ala Ser Asn Asn
145                 150                 155                 160

Leu Phe Ala Cys Leu Pro Cys Thr Ala Cys Lys Ser Asp Glu Glu Glu
                165                 170                 175

Arg Ser Pro Cys Thr Thr Thr Arg Asn Thr Ala Cys Gln Cys Lys Pro
            180                 185                 190

Gly Thr Phe Arg Asn Asp Asn Ser Ala Glu Met Cys Arg Lys Cys Ser
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Thr Gly Cys Pro Arg Gly Met Val Lys Val Lys Asp Cys Thr Pro Trp
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Val Leu Ile Val Cys Cys Cys Ile Gly Ser Gly Cys Gly Gly Asp Pro
            260                 265                 270

Lys Cys Met Asp Arg Val Cys Phe Trp Arg Leu Gly Leu Leu Arg Gly
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Pro Gly Ala Glu Asp Asn Ala His Asn Glu Ile Leu Ser Asn Ala Asp
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Ser Leu Ser Thr Phe Val Ser Glu Gln Gln Met Glu Ser Gln Glu Pro
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Ala Asp Leu Thr Gly Val Thr Val Gln Ser Pro Gly Glu Ala Gln Cys
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Leu Leu Gly Pro Ala Glu Ala Glu Gly Ser Gln Arg Arg Arg Leu Leu
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Thr Ala Gly Pro Gly Asp Ala Leu Tyr Ala Met Leu Met Lys Trp Val
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Asn Lys Thr Gly Arg Asn Ala Ser Ile His Thr Leu Leu Asp Ala Leu
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Glu Arg Met Glu Glu Arg His Ala Lys Glu Lys Ile Gln Asp Leu Leu
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Cys Pro Pro Gly His His Ile Ser Glu Asp Gly Arg Asp Cys Ile Ser
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Cys Lys Tyr Gly Gln Asp Tyr Ser Thr His Trp Asn Asp Leu Leu Phe
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Cys Leu Arg Cys Thr Arg Cys Asp Ser Gly Glu Val Glu Leu Ser Pro
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Cys Thr Thr Thr Arg Asn Thr Val Cys Gln Cys Glu Glu Gly Thr Phe
    130                 135                 140

Arg Glu Glu Asp Ser Pro Glu Met Cys Arg Lys Cys Arg Thr Gly Cys
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Pro Arg Gly Met Val Lys Val Gly Asp Cys Thr Pro Trp Ser Asp Ile
                165                 170                 175

Glu Cys Val His Lys Glu Ser Gly Thr Lys His Ser Gly Glu Ala Pro
            180                 185                 190

Ala Val Glu Glu Thr Val Thr Ser Ser Pro Gly Thr Pro Ala Ser Pro
        195                 200                 205

Cys Ser Leu Ser Gly Ile Ile Ile Gly Val Thr Val Ala Ala Val Val
    210                 215                 220

Leu Ile Val Ala Val Phe Val Cys Lys Ser Leu Leu Trp Lys Lys Val
225                 230                 235                 240

Leu Pro Tyr Leu Lys Gly Ile Cys Ser Gly Gly Gly Gly Asp Pro Glu
                245                 250                 255

Arg Val Asp Arg Ser Ser Gln Arg Pro Gly Ala Glu Asp Asn Val Leu
            260                 265                 270

Asn Glu Ile Val Ser Ile Leu Gln Pro Thr Gln Val Pro Glu Gln Glu
        275                 280                 285

Met Glu Val Gln Glu Pro Ala Glu Pro Thr Gly Val Asn Met Leu Ser
    290                 295                 300

Pro Gly Glu Ser Glu His Leu Leu Glu Pro Ala Glu Ala Glu Arg Ser
305                 310                 315                 320

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Thr Leu Arg Gln Cys Phe Asp Asp Phe Ala Asp Leu Val Pro Phe Asp
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Ser Trp Glu Pro Leu Met Arg Lys Leu Gly Leu Met Asp Asn Glu Ile
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Lys Val Ala Lys Ala Glu Ala Ala Gly His Arg Asp Thr Leu Tyr Thr
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Met Leu Ile Lys Trp Val Asn Lys Thr Gly Arg Asp Ala Ser Val His
385                 390                 395                 400

Thr Leu Leu Asp Ala Leu Glu Thr Leu Gly Glu Arg Leu Ala Lys Gln
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Lys Ile Glu Asp His Leu Leu Ser Ser Gly Lys Phe Met Tyr Leu Glu
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Glu Glu Cys Pro Ala Gly Ser His Arg Ser Glu Tyr Thr Gly Ala Cys
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Asn Pro Cys Thr Glu Gly Val Asp Tyr Thr Ile Ala Ser Asn Asn Leu
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Pro Ser Cys Leu Leu Cys Thr Val Cys Lys Ser Gly Gln Thr Asn Lys
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Ser Ser Cys Thr Thr Thr Arg Asp Thr Val Cys Gln Cys Glu Lys Gly
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Gly Cys Pro Arg Gly Met Val Lys Val Ser Asn Cys Thr Pro Arg Ser
                165                 170                 175

Asp Ile Lys Cys Lys Asn Glu Ser Ala Ala Ser Ser Thr Gly Lys Thr
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        195                 200                 205

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Val Leu Phe Arg Arg Arg Ser Cys Pro Ser Arg Val Pro Gly Ala Glu
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Asp Ala Asp Ser Ala Asp Ile Ser Thr Leu Leu Asp Ala Ser Ala Thr
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Leu Glu Glu Gly His Ala Lys Glu Thr Ile Gln Asp Gln Leu Val Gly
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Val Phe Cys Thr Lys Thr Ser Asp Thr Val Cys Asp Ser Cys Glu Asp
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Ser Thr Tyr Thr Gln Leu Trp Asn Trp Val Pro Glu Cys Leu Ser Cys
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Gly Ser Arg Cys Ser Ser Asp Gln Val Glu Thr Gln Ala Cys Thr Arg
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Glu Gln Asn Arg Ile Cys Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu
        115                 120                 125

Ser Lys Gln Glu Gly Cys Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg
    130                 135                 140

Pro Gly Phe Gly Val Ala Arg Pro Gly Thr Glu Thr Ser Asp Val Val
145                 150                 155                 160

Cys Lys Pro Cys Ala Pro Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr
                165                 170                 175

Asp Ile Cys Arg Pro His Gln Ile Cys Asn Val Val Ala Ile Pro Gly
            180                 185                 190

Asn Ala Ser Arg Asp Ala Val Cys Thr Ser Thr Ser Pro Thr Arg Ser
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Met Ala Pro Gly Ala Val His Leu Pro Gln Pro Val Ser Thr Arg Ser
    210                 215                 220

Gln His Thr Gln Pro Thr Pro Glu Pro Ser Thr Ala Pro Ser Thr Ser
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Leu Leu Ile Ile Gly Val Val Asn Cys Val Ile Met Thr Gln Val Lys
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Lys Lys Pro Leu Cys Leu Gln Arg Glu Ala Lys Val Pro His Leu Pro
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Lys Phe Val Val Val Ile Val Ala Val Leu Leu Pro Val Leu Ala Tyr
50 55 60

Ser Ala Thr Thr Ala Arg Gln Glu Glu Val Pro Gln Gln Thr Val Ala
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Pro Gln Gln Gln Arg His Ser Phe Lys Gly Glu Glu Cys Pro Ala Gly
85 90 95

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Ser His Arg Ser Glu His Thr Gly Ala Cys Asn Pro Cys Thr Glu Gly
            100                 105                 110

Val Asp Tyr Thr Asn Ala Ser Asn Asn Glu Pro Ser Cys Phe Pro Cys
        115                 120                 125

Thr Val Cys Lys Ser Asp Gln Lys His Lys Ser Ser Cys Thr Met Thr
    130                 135                 140

Arg Asp Thr Val Cys Gln Cys Lys Glu Gly Thr Phe Arg Asn Glu Asn
145                 150                 155                 160

Ser Pro Glu Met Cys Arg Lys Cys Ser Arg Cys Pro Ser Gly Glu Val
                165                 170                 175

Gln Val Ser Asn Cys Thr Ser Trp Asp Asp Ile Gln Cys Val Glu Glu
            180                 185                 190

Phe Gly Ala Asn Ala Thr Val Glu Thr Pro Ala Ala Glu Glu Thr Met
        195                 200                 205

Asn Thr Ser Pro Gly Thr Pro Ala Pro Ala Ala Glu Glu Thr Met Asn
    210                 215                 220

Thr Ser Pro Gly Thr Pro Ala Pro Ala Ala Glu Glu Thr Met Thr Thr
225                 230                 235                 240

Ser Pro Gly Thr Pro Ala Pro Ala Ala Glu Glu Thr Met Thr Thr Ser
                245                 250                 255

Pro Gly Thr Pro Ala Pro Ala Ala Glu Glu Thr Met Thr Thr Ser Pro
            260                 265                 270

Gly Thr Pro Ala Ser Ser His Tyr Leu Ser Cys Thr Ile Val Gly Ile
        275                 280                 285

Ile Val Leu Ile Val Leu Leu Ile Val Phe Val
    290                 295
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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C07K 14/715, C12N 15/12, 15/63, C07K 16/28 — A3

(11) International Publication Number: WO 00/34335

(43) International Publication Date: 15 June 2000 (15.06.00)

(21) International Application Number: PCT/US99/26035

(22) International Filing Date: 3 December 1999 (03.12.99)

(30) Priority Data:
09/205,018 4 December 1998 (04.12.98) US

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(74) Agents: SCHRAM, David, B. et al.; Schering–Plough Corporation, Patent Department, K–6–1 1990, 2000 Galloping Hill Road, Kenilworth, NJ 07033–0530 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, HR, HU, ID, IL, IN, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LU, LV, MA, MD, MG, MK, MN, MX, NO, NZ, PL, PT, RO, RU, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(88) Date of publication of the international search report:
10 August 2000 (10.08.00)

(54) Title: "DEATH DOMAIN" CONTAINING RECEPTORS

(57) Abstract

Purified genes from a mammalian virus, reagents related thereto including purified proteins, specific antibodies, and nucleic acids encoding the polypeptides are provided. Methods of using said reagents and diagnostic kits are also provided.

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TD	Chad
TG	Togo
TJ	Tajikistan
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UA	Ukraine
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VN	Viet Nam
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ZW	Zimbabwe

INTERNATIONAL SEARCH REPORT

In: ational Application No
PCT/US 99/26035

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/715 C12N15/63 C07K16/28 C12N15/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 30387 A (AVIRON INC) 3 October 1996 (1996-10-03) SEQ ID NO: 6, nucleotides 8008-8538; SEQ ID NO:18 -/--	1-10, 15-17

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 May 2000	31/05/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Schönwasser, D

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/26035

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHA T.A. ET AL.: "Human Cytomegalovirus Clinical Isolates Carry at Least 19 Genes Not Found in Laboratory Strains" JOURNAL OF VIROLOGY, vol. 70, no. 1, 1996, pages 78-83, XP002137489 cited in the application	1-10, 15-17
X	-& CHA T.A. ET AL.: "Human cytomegalovirus clinical isolates carry at least 19 genes not found in laboratory strains; human cytomegalovirus Toledo strain UL/b' region" EMBL DATABASE ENTRY HC33331; ACCESSION NO. U33331,29 January 1996 (1996-01-29), XP002137490 cited in the application	
P,X	LURAIN N.S. ET AL.: "Human cytomegalovirus UL144 open reading frame: sequence hypervariability in low-passage clinical isolates" JOURNAL OF VIROLOGY, vol. 73, no. 12, December 1999 (1999-12), pages 10040-10050, XP000906955 Fig. 4, first sequence (Toledo)	1-10, 15-17
P,X	-& LURAIN N.S. ET AL.: "Human herpesvirus 5 isolate PTO26UL144 UL144 protein (UL144) gene, complete cds." EMBL DATABASE ENTRY AF085001; ACCESION NO. AF085001,21 November 1999 (1999-11-21), XP002137491	
A	MEINL E ET AL: "Anti-apoptotic strategies of lymphotropic viruses" IMMUNOLOGY TODAY, vol. 19, no. 10, 1 October 1998 (1998-10-01), pages 474-479, XP004142764 ISSN: 0167-5699 the whole document	1-20

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/ 26035

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Insofar as claim 11 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 11b (inc. search)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 11b (incomplete search)

Present claim 11b relates to a method for modulating apoptosis of a cell comprising the exposure of said cell to a sufficient amount of a blocking compound which block the effect of UL144 on apoptosis whereby the claim encompasses an extremely large number of possible blocking compounds. Support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the blocking compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to antibodies which bind UL144 and antisense nucleic acids comprising sequences complementary to a nucleic acid which encodes UL144 (see claim 20).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In ational Application No
PCT/US 99/26035

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9630387 A	03-10-1996	US	5721354 A	24-02-1998
		AU	706234 B	10-06-1999
		AU	5372796 A	16-10-1996
		CA	2215328 A	03-10-1996
		EP	0821693 A	04-02-1998
		JP	11503013 T	23-03-1999
		US	6040170 A	21-03-2000
		US	5925751 A	20-07-1999